AN EXPERIMENTAL CONTRIBUTION TO THE STUDY OF CARDIAC HYPERTROPHY.*

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PLATES VI–IX.

It is seldom that what seems an undoubted and established fact in biology receives universal acceptance. There are nearly always those who find reasons for antagonizing it. Thus, in the subject titled, the view has been held since the days of Corvisart that the stimulus to hypertrophy is increased work on the part of the organ concerned. But there are two prominent investigators who oppose this conception.

Horvath (1) maintains that there is no scientific proof that hypertrophy is due to increased work and that there is no relation between the size of an organ and the amount of work which it has to perform. He points out that the muscles of mastication and the diaphragm are smaller relatively than the amount of work which is demanded of them. The uterus hypertrophies during pregnancy, yet it does not perform work while enlarging.

Horvath's explanation of hypertrophy has its fundamental basis in the acceptance of Fick's law, which holds that muscles elicit more or less work according to the degree to which they are stretched. He believes that hypertrophy can occur only if the stimulus to contraction, or the contraction itself, results in a stretching greater than normal. He sees in this extension a stimulus which has a twofold action. It incites to increased contraction and also to increased growth. These results are related not in the sense of cause and effect, but only in that they have a common origin.

Of an entirely different nature is the hypothesis advanced by Albrecht (2). This author made an extensive histological study of the hypertrophic heart muscle, and his observations have led him to refute the mechanical theory of hypertrophy. This theory, according to Albrecht, will not explain the fact that, in cases of idiopathic hypertrophy, we may find an enormous increase in the size of the heart without any known agent which can increase its work; while, on the other hand, in valvular disease, in which undoubtedly the heart is working under great difficulty, there is never such an extensive enlargement. He

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has found also that the hypertrophic changes may be localized in such different situations of the heart that a functional relationship cannot be held to exist between them. He further supports his contention that hypertrophy is not a compensatory process by the fact that the enlargement of the muscle fiber depends entirely on an increase of its sarcoplasm and not on the contractile elements; viz., the fibrillæ.

Albrecht bases his theories on the following facts. There is uniformly an increase in the amount of connective tissue. The muscle cells are vacuolated, the nuclei often double, and there is a degeneration of the anisotropic, and an increase of the isotropic portion of the muscle elements. He interprets these lesions as being of an inflammatory nature, identical with the hypertrophic conditions of the liver cells which Virchow found in the early stage of inflammation.

In the present article we wish to deal, not with the primary cause of hypertrophy, nor with its mechanism, but with one phase of it: first, the development of the reactive processes known as hypertrophy, in point of view of time after the establishment of a condition favorable to its onset; secondly, the question of co-hypertrophy; and thirdly, the relation which the altered muscle bears to the normal.

METHOD OF INVESTIGATION.

In order to obtain a condition of hypertrophy of the heart, the most certain method is the production of an aortic insufficiency. This procedure has been used by most investigators of this subject, but, whereas previously a sound was used to rupture the aortic valves, in the present work the valvulotome devised by MacCallum (3) was employed. This instrument possesses the distinct advantage over the sound in that the extent of the lesion produced is always the same; namely, a complete division of the cusp. A uniformity in the extent of the lesion is essential since, as was shown by Hasenfeld and Romberg (4), the alteration in the work which the heart has to perform and, presumably, also in the degree of hypertrophy, vary with the extent of the lesion. The puncture is also liable to be completely plugged by fibrin, as was noticed by Hirschfelder (5) and by the author (6). Furthermore, the valvulotome is superior to the sound in so far that any chosen cusp may be destroyed, whereas such a selection is impossible when the latter is used.

In this series of experiments the animals used throughout were dogs. They are especially adapted for this purpose since, by the use of the valvulotome, an aortic insufficiency can be produced with great ease and certainty. The instrument is passed, under aseptic precautions, down the left carotid artery until the point reaches the sinus of Valsalva which lies between the posterior aortic cusp and the anterior mitral segment. It is now passed through the base of the cusp, the cutting edge turned toward the lumen of the aorta, and, on withdrawing it, the cusp is completely divided in two. The lesion is accompanied immediately by the development of the so-called collapsing pulse and a diastolic

murmur. The posterior aortic cusp was selected since neither of the coronary arteries open from the corresponding sinus of Valsalva.

Plate VI, Fig. 1, is a semi-diagrammatic representation of the nature of the lesion produced by the valvulotome. When the insufficiency has lasted any appreciable length of time, the cut edges of the damaged valve become thickened, and the ends not infrequently project into the aorta, as shown in the figure.

The subsequent development of hypertrophy was determined by the use of proportional weights; namely, the comparison of the proportional weight of the heart to the body weight in the normal animal and in the animal in which an aortic lesion had been produced. This method was first used by W. Müller (7), who showed that the human heart bears an almost constant relation to the body weight at the different age periods, and irrespective of the state of general nutrition of the individual. The same constancy in proportional weight has been found to be the most accurate method for a correct determination of the changes in the size of the heart produced experimentally. It was found necessary, in addition to the proportional weight of body to heart, to construct tables showing the relation of the different segments of the heart—left ventricle, right ventricle, septum, and auricles—to the body weight in the normal animal. By means of these figures it is possible to form an estimate of the changes in size of the whole heart, or of a segment of the same, which result from the valvular lesion.

The isolation of the different segments is performed briefly as follows: the epicardial fat is first removed and the vessels are cut close to their attachment to the heart. The auricles are then removed by cutting along the auriculoventricular junction. The separation of the right ventricle from the left is accomplished by cutting along the interventricular grooves anteriorly and posteriorly. In order to have a uniform method for the separation of the septum, which is not so clearly marked off from the left ventricle as is the right, the muscle was cut radially along the incision made for the removal of the right ventricle.

The animals after operation were kept in a separate room with a yard attached and allowed to run about at will. The hearts were examined at periods varying from four days to three months.

THE PROPORTIONAL WEIGHT OF THE NORMAL DOG'S HEART TO ITS BODY WEIGHT.

As has been mentioned above, attention was first called by W. Müller to the close relationship which exists between the weight of the heart and the body weight in man. Taking the body weight as unity, the proportion of the heart weight is 0.00567. From an examination of 1,500 bodies, he was able to show that the male human heart is larger than that of the female, and further, that the weight of the heart increases slightly with age. Müller's results have been amply confirmed by later investigators and his method has been employed for a comparison of the relative size of the heart of animals of different species. Thus Bergmann (8) found that a constancy exists in the animals of the same species, but the ratio varies greatly with animals of different species. So far

as we have been able to gather from the literature, only two tables, those of Heinz (9) and of Joseph (10), have been published, giving the proportional weight[1] of the dog's heart. These authors differ very materially in their results. Heinz found the proportional weight to be 0.0097, while Joseph gives it as 0.0074. Such a disparity is too great to render an average of the figures of value in the determination of hypertrophy. It was therefore found necessary to make an independent series of observations for controls and, as will be shown later, the results accord very closely with those of Joseph; viz. 0.00721. It is not improbable that the differences recorded are due, as Joseph suggests, to a difference of the breed of the animals used for the experimentation in this country and in Germany.

It is unfortunate that neither of these authors gives figures for the sectional weights of the four heart segments, the value of which has been so strongly emphasized by Krehl (11) and by Grober (12) for the detection of hypertrophy of a single chamber. This information for the normal animal is supplied in the present research and gives us a standard whereby the changes in the various segments following aortic insufficiency can be accurately determined.

Table I has been constructed from the weighings of twenty normal dog's hearts and gives the absolute weight in grams of the body and the whole heart and of the four segments with the proportional and sectional weight.

It will be noticed that, notwithstanding the considerable difference in the size of the animals, the proportional weights vary only from 0.00609 to 0.00856, the average being 0.00721. It is necessary at this point to mention that subsequently, when dealing with hypertrophic hearts, we shall not regard a heart as enlarged unless it exceeds the highest value found in the series for the normal, namely, 0.00856, which is much in excess of the average.

Equally constant are the figures which we have obtained for the separate segments. The highest proportional weight of the left ventricle in our series was 0.00358, the lowest was 0.00226, and the average was 0.00315. As in the case of the whole heart, a segment is to be considered hypertrophied only if it exceeds the *highest* re-

[1] It may be stated here that throughout this paper the words "proportional weight" will be frequently used, and they are to be interpreted as an abbreviation for "the ratio which the heart weight bears to body weight." The words "sectional weight" signify the "ratio which the weight of a given segment bears to body weight."

TABLE I.

Proportional and Sectional Weight in the Normal Dog.

Sex.	Body weight in grams.	Heart weight in grams.	Left ventricle weight in grams.	Septum weight in grams.	Right ventricle weight in grams.	Auricular weight in grams.	Total heart weight to body weight.	Left ventricle weight to body weight.	Septum weight to body weight.	Right ventricle weight to body weight.	Auricular weight to body weight.
M.	9,500	80.4	33.9	16.3	20.5	9.7	0.00840	0.00356	0.00171	0.00215	0.00102
M.	7,800	66.8	27.9	15.6	14.6	8.7	0.00856	0.00357	0.00200	0.00189	0.00111
M.	6,000	46.0	20.4	9.0	10.7	5.9	0.00766	0.00340	0.00150	0.00178	0.00098
F.	11,800	70.7	31.8	14.5	14.4	10.0	0.00599	0.00269	0.00122	0.00122	0.00084
F.	21,850	139.1	49.4	35.4	33.9	20.4	0.00636	0.00226	0.00162	0.00155	0.00093
F.	12,800	86.7	38.9	20.4	18.0	9.4	0.00677	0.00303	0.00159	0.00140	0.00073
M.	5,650	38.0	17.5	8.7	7.5	4.3	0.00672	0.00309	0.00153	0.00132	0.00076
M.	11,100	67.7	27.6	14.8	16.4	8.9	0.00609	0.00248	0.00133	0.00147	0.00080
M.	5,500	42.34	18.20	9.57	9.29	5.28	0.00769	0.00330	0.00174	0.00168	0.00096
F.	7,700	57.5	24.2	14.8	12.7	5.8	0.00746	0.00314	0.00192	0.00164	0.00075
M.	5,800	46.1	20.8	11.2	10.2	3.9	0.00794	0.00358	0.00193	0.00175	0.00067
F.	6,000	47.05	22.05	9.25	11.15	4.6	0.00784	0.00367	0.00154	0.00185	0.00076
F.	5,600	42.95	19.95	10.50	8.50	4.0	0.00766	0.00356	0.00187	0.00151	0.00071
M.	7,700	48.90	21.70	11.90	10.20	5.1	0.00635	0.00281	0.00154	0.00132	0.00066
M.	12,400	48.65	38.50	20.25	15.40	10.5	0.00681	0.00311	0.00163	0.00124	0.00084
M.	8,400	63.75	26.55	15.45	14.05	7.70	0.00757	0.00316	0.00183	0.00167	0.00091
M.	7,300	57.75	25.50	17.75	12.20	4.30	0.00791	0.00341	0.00215	0.00167	0.00058
F.	4,700	37.75	16.75	9.75	8.00	3.25	0.00803	0.00356	0.00207	0.00170	0.00069
M.	10,300	64.45	26.85	14.6	16.30	6.70	0.00625	0.00260	0.00141	0.00158	0.00065
F.	6,300	39.60	20.20	7.2	7.80	4.40	0.00628	0.00320	0.00114	0.00123	0.00069
Average for the series.....							0.00721	0.00315	0.00166	0.00158	0.00080

TABLE II.

Proportional and Sectional Weight after Production of Aortic Insufficiency.

No.	Sex.	Duration in days.	Body weight in grams.	Heart weight in grams.	Left ventricle weight in grams.	Septum weight in grams.	Right ventricle weight in grams.	Auricular weight in grams.	Heart weight to body weight.	Left ventricle weight to body weight.	Septum weight to body weight.	Right ventricle weight to body weight	Auricular weight to body weight.
1	F.	20	4,000	36.4	16.5	7.9	5.8	6.2	0.00910	0.00412	0.00197	0.00145	0.00155
2	F.	18	5,600	53.2	27.7	11.3	8.0	6.1	0.00950	0.00494	0.00201	0.00142	0.00108
3	M.	27	6,300	83.3	40.0	18.6	15.71	9.0	0.01322	0.00634	0.00295	0.00249	0.00142
4	M.	5	5,900	49.5	26.1	8.0	8.75	6.65	0.00840	0.00442	0.00135	0.00148	0.00112
5	F.	7	6,300	65.0	38.1	15.4	12.60	8.0	0.01031	0.00604	0.00244	0.00200	0.00126
6	M.	12	6,250	58.52	27.20	14.05	10.47	6.8	0.00936	0.00435	0.00224	0.00167	0.00108
7	M.	11	5,000	62.30	29.80	11.7	11.7	9.1	0.01246	0.00596	0.00234	0.00234	0.00182
8	M.	7	4,500	40.00	17.2	10.8	7.0	5.0	0.00888	0.00382	0.00240	0.00155	0.00111
9	F.	21	3,900	45.3	20.8	10.5	8.3	5.7	0.01161	0.00533	0.00269	0.00212	0.00146
10	M.	7	6,500	68.25	34.75	13.3	10.6	9.6	0.01050	0.00534	0.00204	0.00163	0.00147
11	M.	24	3,900	42.05	21.8	7.9	6.55	3.9	0.01078	0.00558	0.00202	0.00167	0.00100
12	F.	18	4,900	38.40	18.9	8.6	6.40	4.5	0.00783	0.00385	0.00175	0.00130	0.00092
13	F.	17	5,300	36.0	18.1	9.7	5.00	3.2	0.00678	0.00341	0.00182	0.00094	0.00060
14	F.	7	5,400	51.7	23.4	12.5	10.9	4.9	0.00957	0.00451	0.00231	0.00201	0.00090
15	M.	24	3,700	51.0	26.6	11.0	8.4	5.0	0.01378	0.00718	0.00297	0.00227	0.00135
16	M.	28	3,300	38.5	17.2	9.8	7.9	3.6	0.01166	0.00521	0.00296	0.00239	0.00109
17	M.	28	4,900	42.5	21.5	8.45	5.8	6.8	0.00867	0.00438	0.00174	0.00118	0.00138
18	M.	56	4,900	52.4	28.0	10.5	8.4	5.5	0.01069	0.00571	0.00214	0.00171	0.00112
19	M.	12	6,000	60.18	29.70	13.08	10.50	6.90	0.01003	0.00495	0.00218	0.00175	0.00115
20	M.	6	8,100	80.59	39.69	17.49	14.02	9.39	0.00999	0.00490	0.00216	0.00173	0.00116
Averages for the series.....									0.01010	0.00501	0.00222	0.00175	0.00120

corded figure for the normal. The average proportional weight for the septum is 0.00166; for the right ventricle, 0.00158; and for the auricles, 0.00080.

THE PROPORTIONAL AND SECTIONAL WEIGHTS AFTER THE PRODUCTION OF AORTIC INSUFFICIENCY.

In table II are given the results of twenty experiments in which the posterior aortic cusp was divided and the animals were allowed to live for varying periods of time.

If, irrespective of the duration of the lesion, an average be taken of the proportional weights of the series, it will be noticed that there is an increase from 0.00721 to 0.01010 in the total heart. The heart is approximately one-third larger than normal. As would be expected, this increase, as shown by the sectional weights, is mainly in the left ventricle and septum, but relatively there is seen to be a slight increase in the weight of the right ventricle and a distinct increase in the weight of the auricles. This point will be referred to in some detail later. The average increase per cent., as shown in table III, is 40 for the total heart, 59.3 for the left ventricle, 33.7 for the septum, 10.7 for the right ventricle, and 50 for the auricles.

TABLE III.

Comparison of Proportional and Sectional Weights in the Normal Animal and after the Production of Aortic Insufficiency.

	Heart weight to body weight.	Left ventricle weight to body weight.	Septum weight to body weight.	Right ventricle weight to body weight.	Auricular weight to body weight.
Normal heart.....	0.00721	0.00315	0.00166	0.00158	0.00080
Hypertrophied heart ..	0.01010	0.00501	0.00222	0.00175	0.00120
Increase per cent.	40.0	59.3	33.7	10.7	50.0

Heretofore, an explanation of the cause of hypertrophy of the left ventricle in aortic insufficiency has presented no difficulties in its acceptance—it was assumed to be an indirect result of regurgitation. Cohnheim's (13) and Rosenbach's (14) original statements have been accepted without modification. Because of the insufficiency of the aortic valves, a part of the blood thrown out during systole returns to the ventricle during diastole and the following systolic output is augmented by exactly this amount. The work performed by the ventricle being roughly the product of the systolic output and the resistance in the aorta, it is evident that, within certain limits, the greater the systolic output,

the greater the work performed. To the extra work, assumed to exist in aortic insufficiency following the increased systolic output, was attributed the stimulant to hypertrophy. This explanation was rendered the more plausible since a parallel could be drawn in the effects produced by insufficiency of the mitral and tricuspid valves. In both these lesions, the effect of the increased systolic output is to cause a hypertrophy of the chamber involved. Accepting the axiom that hypertrophy has its fundamental basis in increased work, we are still compelled to modify the hypothesis originated by Cohnheim and Rosenbach in view of the fact that it has been shown experimentally that only a small amount of blood can regurgitate even in the most extensive insufficiency.

In a former paper (15), the author expressed the opinion that hypertrophy of the left ventricle in insufficiency of the aortic valves results from the increased work induced by the stimulating effect of tension on the ventricular walls during diastole. It is unnecessary to repeat here the arguments in support of this view. It is sufficient to state that it was shown that tension applied to the ventricular wall during diastole (as occurs in aortic insufficiency), is a most potent factor in increasing the force of systolic contraction and, consequently, the work of the left ventricle. The hypothesis of Horvath assumed that tension in itself was the direct cause of hypertrophy, but we have no proof as yet that tension alone will cause hypertrophy. It can do so only if, at the same time, it augments the activities of the muscle upon which it acts.

The association of hypertrophy with a lesion of the aortic valve is so constant a feature that it is difficult, at first sight, to explain the absence of hypertrophy in a case in which an undoubted insufficiency of the aortic valve has been produced. It has, however, been pointed out by Krehl (16) that such indeed can occur, and his observations are confirmed by our experiments. In animals 12 and 13, it was found that instead of hypertrophy following the lesion, the hearts were somewhat smaller than the average for the normal; viz. 0.00783 and 0.00678.

It has already been pointed out in a previous paper (15) that a regulating mechanism is called into play after the production of aortic insufficiency, whereby the increased work thrown on the heart during diastole can be minimized by the lowering of the diastolic blood pressure from dilatation of the peripheral arteries. This self-regulation, in accordance with the general physiologic phenomena, is likely to vary in its completeness, and it is possible that the absence of hypertrophy in cases of aortic insufficiency is to be explained by assuming that the self-regulating mechanism is so complete that little or no additional work is required of the heart.

While the explanation of hypertrophy of the left ventricle, given above, seems rational in view of the recent facts which have come to light concerning the pathological physiology of aortic insufficiency, yet it must be admitted that it is not in itself complete, for we have still to account for the hypertrophy, in varying degrees, of all the cardiac segments which results from insufficiency of the aortic valves alone. Table III shows that the interventricular septum increased 33.7 per cent. in weight after the production of aortic insufficiency. This increase may be accounted for by the fact that the septum contains fibers belonging to both the right and left ventricles and that only the latter have undergone hypertrophy. After aortic insufficiency, the section weight of the left ventricle amounts to 0.00501 as compared with 0.00315 in the normal—an increase of 59.3 per cent.—while the corresponding increase, as already mentioned in the case of the septum, is only 33.7 per cent. The degree of hypertrophy is thus roughly proportional to the number of fibers acted upon by the diastolic tension. In a similar way, the slight increase in the right ventricle, 10.7 per cent., may be attributed to the involvement of those fibers common to both chambers.

But how are we to account for the very distinct hypertrophy which our figures show exists in the auricles? The normal sectional weight of the auricles in our series is 0.00080, but, after the production of aortic insufficiency, this proportion is seen to rise to 0.00120, an increase of 50 per cent. Previous work in other animals makes it beyond dispute that the facts have been correctly observed. It was found by Hasenfeld and Romberg (18) in a large percentage of their rabbits, and the protocols of Stadler (19), who also used rabbits, show a hypertrophy of the auricles in all of his animals except one.

The question could be most effectively and simply answered on the assumption that the hypertrophy of the chamber is the result of a relative mitral insufficiency. It has already been shown experimentally that a mitral insufficiency can very readily develop and pass off again during the course of a single experiment. These transient insufficiencies are now known to be caused by a loss of ventricular tone. But in our experiments, every animal was care-

fully auscultated for evidence of mitral insufficiency, and it was only in two cases that a mitral systolic murmur could be detected. After opening the chest of several of our animals, the heart was ausculted directly, but with negative results as far as a mitral murmur was concerned.

We also observed the condition of the auricles, especially the left one, with a view to determine whether they were unduly distended. In none of our animals was there evidence of distension such as would be caused by an increased auricular pressure. We must conclude, therefore, that an explanation other than mitral insufficiency must be sought for the hypertrophy of the auricles in aortic insufficiency.

Rosenbach (20) and Senator (21) explained the hypertrophy of the auricles and right ventricle on the assumption that the more actively contracting left ventricle drives more blood into the coronary arteries and, as a result of the increased nutriment thus conveyed to the heart, the musculature undergoes hypertrophy. It is unnecessary to comment further on this hypothesis as it is now well known that a mere increase in blood supply will not of itself cause hypertrophy.

Hasenfeld and Romberg, in seeking to explain this condition, discuss two possibilities. The left ventricle may not be able to dilate sufficiently to accommodate the blood which has regurgitated from the aorta together with the normal quantity from the auricle, the outflow from the latter is impeded in this way and the work of the left auricle is thus increased. This hypothesis was tested experimentally on the models of von Basch (22) and of Moritz (23). In von Basch's model, after the production of an insufficiency, "the left ventricle" could not expand sufficiently and there immediately followed "a diminished outflow from the left auricle." This, however, did not occur if the distensibility of the "left ventricle" was increased as in Moritz' model.

On analysis this argument is found to be insufficient to account for the facts. Apart from the consideration that it is based on experiments with models which, however intricately constructed, cannot be made to simulate the adaptability of the heart to the varying external and internal conditions, it is possible to adduce evidence that will directly controvert this argument. In the first place, the amount of regurgitated blood in a case of complete section of an aortic cusp does not amount to more than a fraction of a cubic centimeter of blood. This was first proved by the author (24) and was subsequently confirmed by Zollinger (25) by plethysmographic records of the ventricles. After the production of aortic insufficiency, if the tone of the ventricles is maintained, there is only a very slight increase, never amounting to one cubic centimeter of blood, in the amount of systolic output of the ventricles. So small an amount could in no way impede the outflow from the auricle. The only conceivable factor which could obstruct the outflow from the auricle is the positive pressure

in the ventricle transmitted from the aorta during diastole. But it has already been shown in the work of Henderson (26) that normally the ventricle has filled in the early phase of diastole, and the filling, therefore, must occur with very great rapidity. Notwithstanding the difference between the pressure in the left auricle and in the aorta, the great disparity between the auriculo-ventricular opening and that produced by division of an aortic valve, will allow the ventricle to be filled normally from the auricle before any appreciable pressure can be transmitted from the aorta. Furthermore, it has not yet been shown whether there is a definite rise of auricular pressure after the production of aortic insufficiency. On the other hand, Hasenfeld and Romberg bring up the question as to whether the overstretching of the ventricular wall may interfere with the completeness of auricular systole, the increased amount of residual blood in the ventricle impeding the normal outflow from the auricles. That this argument is irrelevant is proved by the fact that it has been shown that there is no increase of residual blood in the left ventricle after insufficiency. On the contrary, the amount of the residual blood is less than normal.

It would appear, then, as if a solution of the problem of the concomitant hypertrophy of the auricles must be sought for in an entirely different direction, and with this aim in view a series of experiments was performed in order to ascertain the cause of auricular co-hypertrophy.

THE CAUSE OF AURICULAR CO-HYPERTROPHY.

From the work of Hirsch (27) and others, we know that the hypertrophy of chronic interstitial nephritis involves all the chambers of the heart. Without entering into a discussion of the causes which increase the work of the heart in this condition (an increased peripheral resistance or an increased viscosity of the blood), it is evident that these causes are operative primarily on the left ventricle and the arterioles. No pathological factor has been discovered which, in this disease, can augment the work of the auricles. In the same way, the cardiac hypertrophy associated with arteriosclerosis without valvular disease, while more especially affecting the left ventricle, is present also in the auricles, as is shown in the table which has been taken from Hirsch's (27) work.

In no way can it be held that, in the hypertrophy of chronic nephritis and arteriosclerosis, the passage of blood from the auricles to the ventricles is impeded to such an extent that the attendant hypertrophy of the auricles is to be explained by this factor. From a study of the hypertrophy under these conditions we have indirect

TABLE IV.

No.	Body weight in grams.	Heart weight in grams.	Auricular weight in grams.	Right ventricle weight in grams.	Left ventricle weight in grams.	Septum weight in grams.
7	46	258 (230.2)	46 (44)	56 (47.1)	87 (84.5)	69 (63.2)
8	93	263 (376.3)	56 (50.4)	46.5	86.5	74
9	60	277 (264)	50 (44.9)	60 (49.7)	107 (92.7)	60 (65.9)
10	37	355 (193.3)	43 (35.1)	55 (40.4)	164 (75.7)	93 (54.7)
11	51	376.5 (264.2)	46 (50.4)	64 (55.6)	156.5 (103.4)	111 (73.9)
12	59	244.5 (264.3)	49.5 (55.6)	44.5 (55.6)	90.5 (103.4)	60 (73.9)
13	29	215 (133.5)	41.5 (31.5)	41 (28.9)	76.5 (52.9)	56 (40.3)
14	39	245 (193.3)	47 (31.5)	42.5 (37.7)	91.5 (66.8)	64 (50.4)
15	42	246 (230)	39 (39.4)	52 (47.1)	95 (84.5)	60 (63.2)
16	49	316 (230.2)	42 (39.4)	101.5 (47.1)	96.5 (84.5)	76 (63.2)
17	60	570 (264.3)	74 (44)	84 (55.6)	251 (103.4)	161 (73.9)
18	45	267 (230)	49 (36.9)	56 (41.9)	101 (79.9)	60 (57.5)
19	71	334 (322.3)	55 (50.4)	45 (66.1)	163 (131.3)	71 (90.5)
20	44	216 (230.2)	39.5 (31.5)	45 (41.9)	74 (79.9)	57.5 (57.5)

The figures in parenthesis represent the normal weight (for the corresponding segment) for the given body weight.

evidence that any condition which augments the work of the left ventricle alone can cause hypertrophy of the auricles without, at the same time, altering the dynamics of the circulation whereby an impediment is offered to their normal action. If our fundamental conception of the cause of hypertrophy, namely, that it is the result of increased work, is correct, we must infer that, in such conditions as those described above, there is an increase in the force of auricular contraction without any discoverable change in the relations of the venous and arterial pressure such as would augment the work demanded of them. This inference has been subjected to an experimental test in the following series of experiments.

THE EFFECT OF INCREASED VENTRICULAR CONTRACTION ON AURICULAR CONTRACTION.

Two methods have been adopted to increase primarily the force of ventricular contraction, namely, compression of the thoracic aorta and the production of aortic insufficiency. In the first condition, the stimulus which incites the left ventricle to increased work is the augmented resistance offered to the systolic output. In the case of aortic insufficiency, the stimulus lies in the transmission to the walls of the ventricle of the diastolic pressure in the aorta.

In plate VII, Fig. 2, a simultaneous record was taken of the contractions of the right auricle, by the suspension method, and of the blood pressure in the right carotid artery. At the point marked *X*, the thoracic aorta was compressed in order to increase the resistance to be overcome by the left ventricle during systole. The blood pressure, of course, rose distinctly, but the record shows that the amplitude of the auricular contractions also underwent an augmentation. It is to be noted that after the relief of pressure, when presumably the left ventricle was beating less forcibly than it did before compression, the auricular contractions became diminished in extent; with the recovery of the left ventricular contractions and rise of blood pressure, the auricular contractions gradually became stronger.

That there is no causal relationship between the rise of arterial blood pressure *per se* and the force of auricular contraction is shown in plate VIII, Fig. 3. The upper curve *A* is that of the auricular contractions; *C* is carotid blood pressure; *B*, base line; *T*, time in seconds; and the lowest curve, *VP*, is the tracing of the right auricular blood pressure measured from the jugular vein. At the point marked *O*, the thoracic aorta was compressed and there was an attendant rise in arterial blood pressure together with an increased force of auricular contraction. The curve *VP*, however, shows that there was no change whatever in the intraauricular blood pressure. This tracing is of special interest also in that it shows the relations between a diminished ventricular and auricular systole and the intraauricular blood pressure. At the point *X*, the vagi were pinched and the effect was to lessen the force of both auricular and ventricular systole without any special change in rate—the inotrope effect of Engelmann. This is seen to give rise to a distinct increase of venous pressure. It will be noticed that the increase in amplitude of the auricular tracing is almost simultaneous with the rise of aortic pressure and is maintained as long as the aorta is compressed. It is, however, necessary to mention that with a prolonged compression of the aorta, as pointed out by Bradford and Dean (28), the ventricle becomes exhausted and the arterial blood pressure falls, and as a result there is a "back" effect on the venous flow which raises the pressure in the right auricle. In such

a short compression as we have used in our experiments, such a condition is not established. The above experiment, then, proves that the increased auricular contractions are independent of changes in venous pressure.

It has been held that such an increased resistance to the systolic output as occurs in chronic nephritis may account for the concomitant hypertrophy of the right ventricle and the auricles, in that there is an incomplete systole of the left ventricle and the increased residual blood dams back on the left auricle, thereby raising the pressure in the pulmonary circulation and the work of the right ventricle. This in turn is assumed to embarrass the right ventricle to such an extent that its residual blood is also increased and, as a consequence, there is a rise in the pressure of the right auricle. That such an explanation is entirely erroneous is proved by the experiments of Bradford and Dean, who showed, as already mentioned, that there is no "back effect" on the pulmonary artery after the compression of the thoracic aorta unless compression is prolonged to the point of exhaustion of the left ventricle.

The effect on the right auricular contraction of an increased systole of the left ventricle can also be studied by the production of aortic insufficiency. In this condition, as previously explained, the stimulus to increased contraction is the diastolic tension of the aorta transmitted to the ventricle in diastole.

Plate IX, Fig. 4 shows the effect on the contractions of the right auricle of an insufficiency of the aortic walls. The upper curve is the auricular contractions, and the blood pressure tracing is taken from the right carotid. At the arrow, the posterior aortic cusp was divided. The irregularity in the auricular curve previous to rupture of the valves results from extra systoles induced by the placing of the point of the valvulotome in the sinus of Valsalva. The immediate effect of rupture of the valve is a rapid fall in pressure, and we have invariably found that this sudden drop is associated with a complete stoppage of the ventricle for a second or two. There is a rapid recovery of the blood pressure to a level somewhat below the original, and the pulsations assume the character of the well-known collapsing pulse of Corrigan.

The auricular contractions show an augmentation similar to that

which occurs during aortic compression. These results have been verified in six different experiments.

To summarize our results, we find that when the left ventricle is incited to increased contraction, there is produced an increased contraction of the right auricle, and also that the increased contractions of the auricle are not associated with a change in auricular pressure.

We cannot at the present time offer an explanation of the physiological mechanism whereby the auricles increase the force of the contractions in sympathy with those of the left ventricle—the subject is at present under investigation and the results will be published shortly. So far as we have gone, we have obtained strong presumptive evidence that there exists an intracardiac reflex which maintains a coördination in the force of systolic contraction between the chambers of the heart.

We have, however, from these results a satisfactory explanation of the cause of auricular co-hypertrophy, provided, of course, that our original premise, that increased work is the stimulus to hypertrophy, is correct.

THE RELATION OF THE ONSET OF HYPERTROPHY TO THE DURATION OF THE LESION.

Tangl (29) thinks that he has adduced evidence from his experiments that the heart does not begin to hypertrophy immediately after the production of an aortic lesion, but only dilates, the increased work being undertaken by the reserve power of the heart until hypertrophy is established. It has, however, been shown experimentally in a previous paper, that beyond a momentary dilatation following the production of the aortic lesion, the effect of a destruction of an aortic cusp is to diminish the amount of residual blood in the left ventricle at the end of the systole. It was then shown that this was accounted for by the stimulating effect of tension of the ventricular walls during diastole. This in itself, while not absolute proof, would tend to indicate that hypertrophy is a precursor to dilatation. Tangl found that only after a certain period, nineteen days, does the musculature of the ventricles undergo dilatation. We have no accurate method for determining whether the auricles or the ventricles show dilatation, since in normal animals the heart at death may be found widely dilated or contracted, the state of contraction or dilatation being determined solely by the stoppage of the heart either in diastole or systole. It is of importance, therefore, to know how soon, after the increased work is thrown on it, the heart gives evidence of the reaction of hypertrophy.

In planning the present series of experiments, we had taken for granted the correctness of Tangl's statements; namely, that a period of at least three weeks was necessary for the establishment of definite signs of hypertrophy, and, in the earlier experiments, our animals were not examined until after the lapse of this period. In the case of one animal which died at the end of one week, we were surprised to find that the proportional weight of the heart showed a very marked increase above the normal. This induced us to kill several of the animals at gradually shorter periods until we could arrive at the point where the enlargement began to show itself. The results are given in chronological order in table V.

TABLE V.

The Relation of the Extent of Hypertrophy to the Duration of the Lesion.

A. First week.

No.	Duration of lesion in days.	Proportional weight.
4	5	0.00840
20	6	0.00999
8	7	0.00888
14	7	0.00957
5	7	0.01031
10	7	0.01050
Average		0.00960
Average increase per cent.		33.1

B. Second and third weeks.

7	11	0.01246
6	12	0.00936
13	17	0.00678 (no hypertrophy)
2	18	0.00950
12	18	0.00783 (no hypertrophy)
1	20	0.00910
9	21	0.01161
Average		0.00952
Average increase per cent.		32.0

C. Three weeks and over.

11	24	0.01078
15	24	0.01378
3	27	0.01322
16	28	0.01166
17	28	0.00867
18	56	0.01069
19	120	0.01003
Average		0.01126
Average increase per cent.		53.5

Of special interest is the remarkable rapidity with which the heart hypertrophies after the production of aortic insufficiency. This hypertropy is well established after one week, and at the end of five days there is sufficient evidence from the tables to show that even then the enlargement is in progress. It will be recalled that Tangl was unable to detect any enlargement in the rabbit's heart before the nineteenth day, although he admitted that he examined only two animals at a shorter period than this. Further, a comparison of the duration of the insufficiency with the increase in average weight shows that up to the seventh day following the insufficiency, there is as much enlargment as at the end of the twentieth day. Taking 0.00721 as the average proportion of the heart weight in the dog, a comparison of the figures in table V shows that an insufficiency lasting seven days raises this proportion to 0.00960, an increase of 33.1 per cent.; while in seven animals, in which the lesion existed from eleven to twenty-one days, there is a proportional weight of 0.00952, an increase of 32 per cent. The increase in the two cases is so close as to be regarded as equal. Beyond the twenty-first day, however, the corresponding proportion has increased to 0.01126, an increase of 56.1 per cent. above the normal.

It would seem that there are two phases in the growth of the hypertrophic heart of the dog, the first occurring during the first week of the conditions favorable to its development, and the other after the third week. There is a period during the second week in which it is stationary as regards growth. It is difficult to conceive that the very great rapidity of growth in the early stage of hypertrophy is an identical condition with that which takes place in the developing heart. An endeavor was made to obtain, from the literature, figures bearing on the rate of growth in the embryonic heart which would serve as a standard for comparison with hypertrophic growth. But the attempt was not followed with success. We know, however, from the careful histological work of Tangl, that the tissue changes in the hypertrophic heart muscle are identical with the changes which are to be recognized in the physiological growth of the heart. His curves show in a convincing manner that, both in physiological and in hypertrophic growth, the size of the individual fibres is proportional to the size of the organ. But

it must be recalled that Tangl's observations were made not earlier than the nineteenth day, by which time, as our experiments show, there is a change in the rate of growth.

To this stage of hypertrophy must also have belonged the hearts examined chemically by Bence (30) and by Gerhartz (31). They came to the conclusion that there is no discernible difference in the chemical composition or in the organic elements of the normal and the hypertrophic heart.

In endeavoring to account for this phenomenon, it occurred to the author that its explanation might be forthcoming in the application of the important discovery made by Ranke (32). He found that after fatigue the molecular concentration of a muscle changes, whereby the blood loses water and gains in solids, while the muscle, on the other hand, takes up water and becomes poorer in solids to a proportional degree. Ranke attributed the increased absorption of water after fatigue to an increased power of imbibition due to the presence of dissociated products of contraction. The application of the laws of physical chemistry to biology by Loeb (33), Overton (34), Höber (35), and others, has resulted in the interpretation of the increased imbibition as an increase of the osmotic pressure within the muscle as a result of fatigue. While Ranke's explanation has been clothed in a modern nomenclature, the substantial truth of his observations has remained unquestioned and has been amply confirmed by a number of recent investigators, among whom may be mentioned Sabbatani (36), Elizabeth Cooke (37), and Fletcher (38). As this increased power of imbibition following activity has not been proved for cardiac muscle, an investigation was made of the water content of the hypertrophied muscle, especially in the early stages of the process, in order to determine whether the increase in volume of the heart after aortic insufficiency could be ascribed to this condition. The results of the analyses are given in the following table.

It will be recalled that Gerhartz had previously found no change in the organic constituent of hypertrophied heart muscle, and an examination of his protocol shows that in the water content also there is no appreciable difference between it and the normal. In our results it will be noticed that in the hypertrophic heart of over

TABLE VI.

Water Content of Normal and Hypertrophied Heart Muscle.

A. Controls: normal hearts.

Heart weight in grams.	Proportional weight.	Weight of dried substance in grams.	Percentage of water.
39.60	0.00628	8.936	77.5
64.45	0.00625	13.964	78.4
46.05	0.00767	9.807	78.7
46.15	0.00796	9.621	79.0
49.80	0.00691	11.000	78.0
42.95	0.00767	9.600	77.7
Average.....	0.00712		78.2

B. Hypertrophied hearts. Duration of lesion five to seven days.

Duration in days.	Heart weight in grams.	Proportional weight.	Weight of dried substance in grams.	Percentage of water.
5	49.5	0.00840	8.41	83.0
6	53.46	0.00999	9.45	82.5
7	40.0	0.00888	7.32	81.7
7	51.7	0.00957	9.25	82.1
7	65.0	0.01031	11.57	82.2
Average.....		0.00943		82.3

C. Hypertrophied hearts. Duration over three weeks.

Duration in days.	Heart weight in grams.	Proportional weight.	Weight of dried substance in grams.	Percentage of water.
24	51.0	0.01378	10.65	79.1
56	52.4	0.01069	10.58	79.8
24	42.05	0.01078	8.62	79.5
11	62.30	0.01246	12.35	80.1
21	45.30	0.01161	9.10	79.9
16	68.25	0.01050	12.91	81.0
Average.....		0.01164		79.9

D. Comparison of results.

	Proportional weight.	Increase per cent. in weight.	Percentage of water.	Increase per cent. of water.
Normal.....	0.00712		78.2	
Early hypertrophy.....	0.00943	32.4	82.3	4.1
Late hypertrophy.....	0.01164	63.6	79.9	1.7

three weeks duration there is a corroberation of Gerhartz's observation.

In table VI are given (1) the weight of the moist heart; (2) the proportional weight of the heart, in order to indicate the degree of

hypertrophy present; (3) the weight of the dried heart substance; (4) the percentage of water.

In table VI, under A, are given the figures for normal hearts; under B, the corresponding figures for the hypertrophic heart for lesions of seven days or less duration; and under C, similar figures for lesions of over eight days duration. These figures clearly bear out the fact that there is a distinct, although small, increase in the water content of the early hypertrophic heart. In the normal heart our results show an average of 78.2 per cent. of water, while up to the eighth day following aortic insufficiency the average for five hearts is 82.3 per cent., an increase of 4.1 per cent. in the amount of water. However, where the lesion has been of longer duration than eight days, the water content has fallen to 79.9, so close to normal as to render the difference negligible.

How then are these results to be interpreted? Is a difference of 4.1 per cent. distinct enough to justify us in concluding that there is an increased absorption of water in the early stage of hypertrophy? The small variation in the individual figures in the respective conditions, and the fact that very great care was taken to prepare each heart for chemical examination in such a way as to preclude any possibility of an error arising from this source, convinces the author that the figures represent the actual condition of affairs. It is necessary, however, to enquire whether the increased absorption of water will account for the total increase of weight of the hypertrophic heart at this stage. This can be ascertained approximately by a comparison of columns 2 and 4 of controls with the corresponding columns in table VI, B. The proportional weight of the hypertrophied hearts amounts to 0.00934, an increase of 32.4 per cent., while in the case of the water content of the same hearts, the increase is only 4.1 per cent. (table VI, D). These results, then, can be interpreted in only one way; namely, that the increase in weight of the heart after the production of aortic insufficiency is not dependent entirely on an absorption of water.

The discovery of the increased power of muscle to imbibe water after activity and fatigue, induced Loeb (39) to advance the theory that the process of hypertrophy is dependent upon an increased osmotic pressure. Loeb holds that the absorption of water is the

primary process in the mechanism of hypertrophy. The stretching and increase in volume of the protoplasm of the muscle fibres which result from this is followed by a deposition of new material. That there is an altered relation between the intake and the output of water in the overacting heart, as well as in the skeletal muscle, seems certain from our results, but from the present state of our knowledge, it is impossible to say whether or not this is the primary process of hypertrophy. It will be recalled, however, that Davenport (40) studied the water content of frogs' eggs at different periods after the commencement of their development and found that growth after the first fourteen days consisted almost entirely of an increase in water. During this period the water content increases from 56 to 96 per cent. and gradually diminishes again with the active formation of protoplasmic substance. While, therefore, as mentioned above, the increase in the weight of the heart in the early stage of hypertrophy is due only in part to an increased water content, it would seem that the altered intake and output of water has an important bearing on both physiologic and hypertrophic growth.

CONCLUSIONS.

1. Hypertrophy induced by the production of aortic insufficiency involves all the chambers of the heart. The greatest absolute increase in weight is in the left ventricle. The remaining segments, arranged in the order of decreasing increments are septum, right ventricle, and auricles. The greatest relative increase is also in the left ventricle, but the auricles show a relative hypertrophy greater than that of the septum or right ventricle.

2. The co-hypertrophy of the auricles is not due to an altered venous pressure, but results from an increased force of auricular systole.

3. The heart shows an increase in weight within one week after the production of aortic insufficiency. The increase in weight is due only in part to an increased water content.

The writer has pleasure in acknowledging the valuable assistance given him by Mr. Melvin Herzfeld in the carrying out this research.

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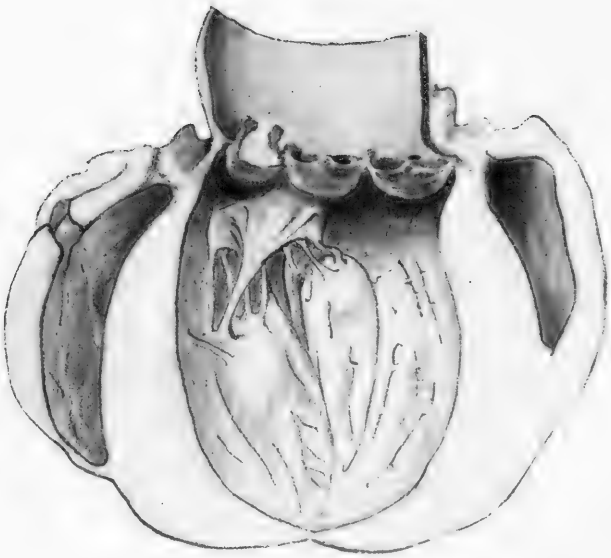

FIG. 1.

UofM

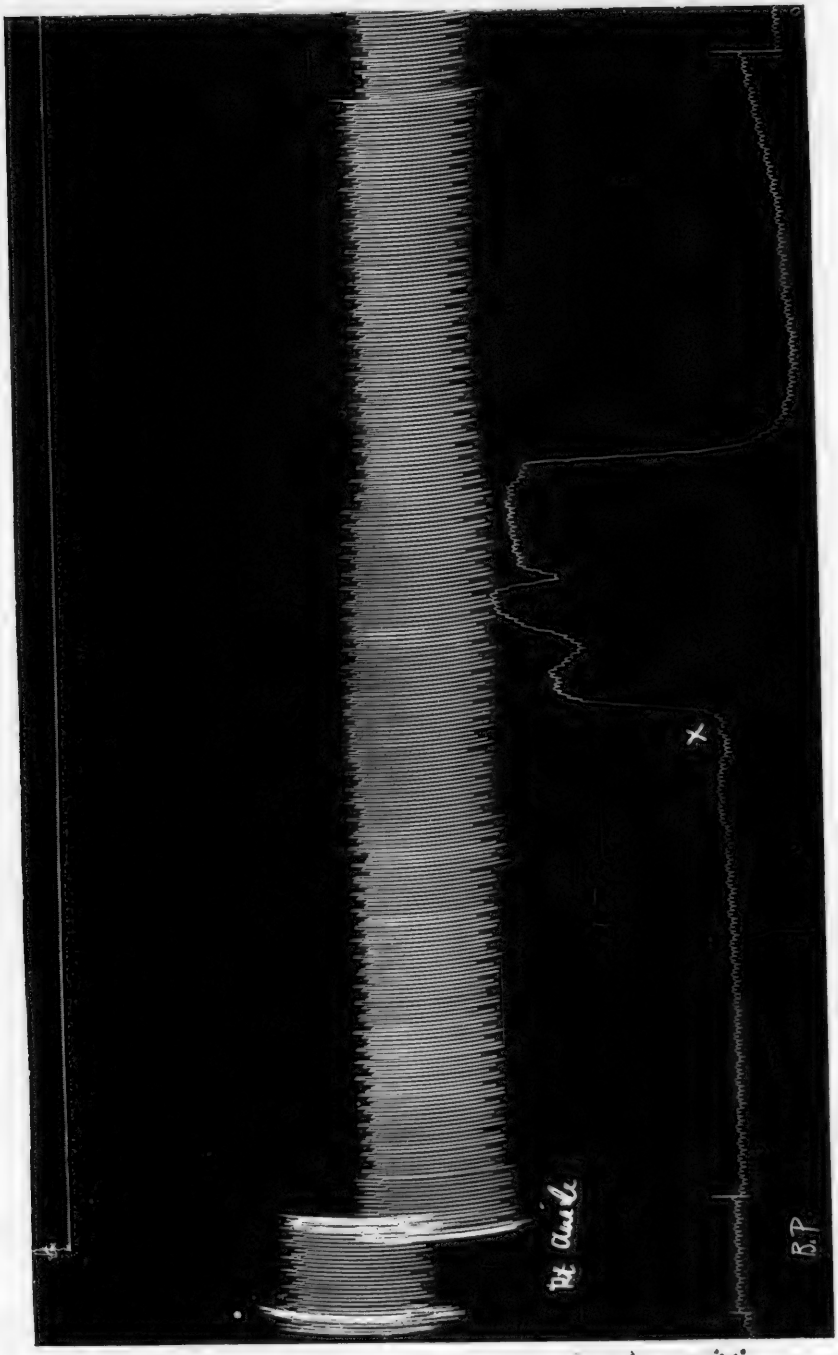

FIG. 2.

Mau

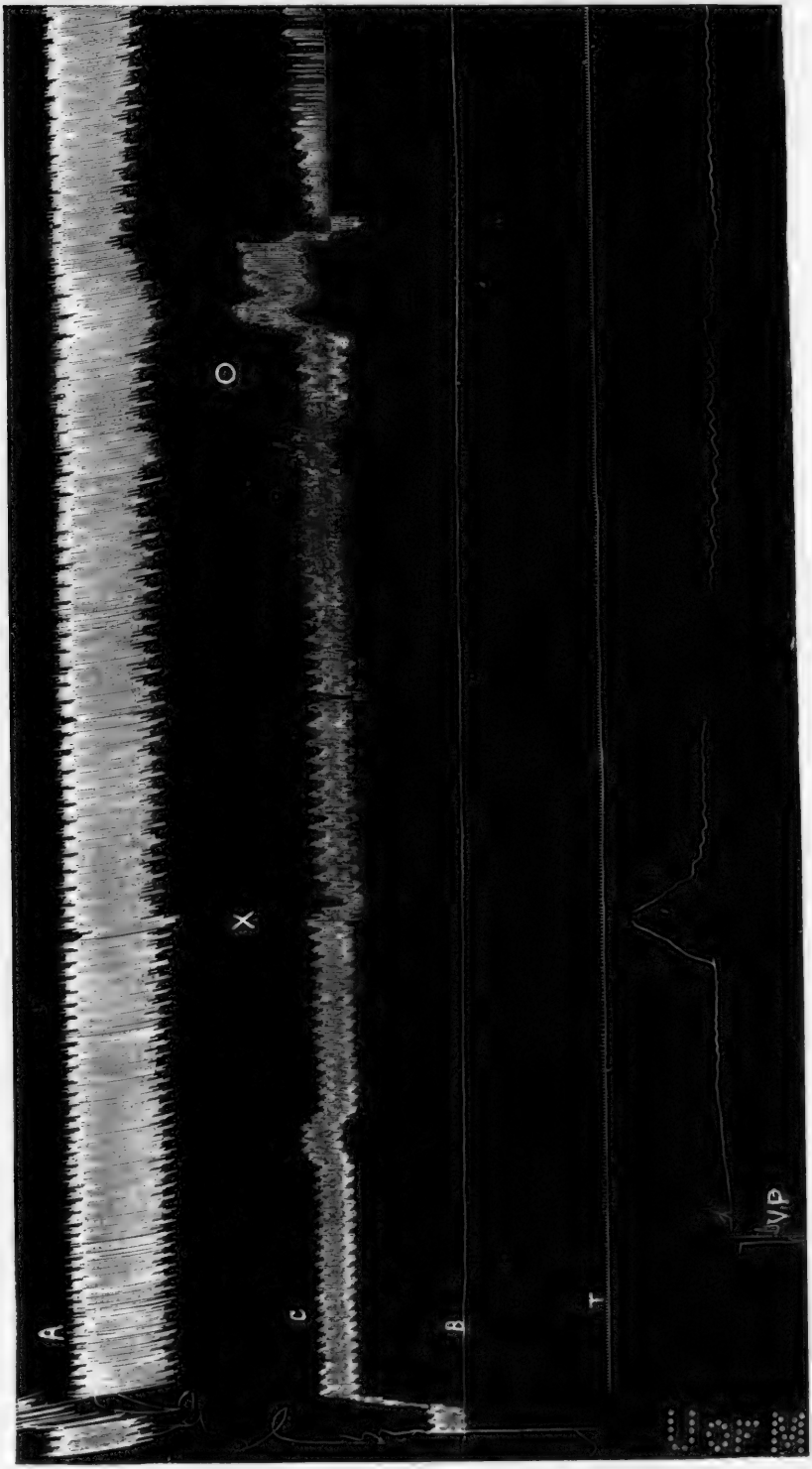

FIG. 3.

FIG. 4.

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EXPLANATION OF PLATES.

Plate VI.

Fig. 1. A semi-diagrammatic representation of the nature of the lesion produced by the valvulotome.

Plate VII.

Fig. 2. Effect of aortic compression. Note increase of auricular contractions during compression and the diminution in size after the compression is relieved.

Plate VIII.

Fig. 3. Effect of aortic compression on auricular contractions, blood pressure, and right auricular pressure. A = right auricular contractions; C = carotid blood pressure; B = base line; T = time in seconds; VP = right auricular blood pressure. At X, vagi were pinched and produced a *weakening* of ventricular and auricular contractions with *rise* in venous pressure. At O, aorta was compressed. Note increase of auricular contractions with no rise in right auricular pressure.

Plate IX.

Fig. 4. Production of aortic insufficiency. RA = right auricular contractions; C = carotid blood pressure. Insufficiency produced at →. Note increase in auricular contractions.

SUBLIMATE AND THE SERUM DIAGNOSIS OF SYPHILIS.*

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According to the studies of those who have been employing the antisheep hemolytic system of Wassermann, the serum reaction of syphilis is apt to disappear suddenly or become very weak shortly after the commencement of mercurial treatment, while the clinical aspect of the patient shows no improvement. This rapid disappearance of the reaction, although not very frequently encountered, renders the value of the Wassermann reaction quite uncertain as a prognostic measure in the treatment of syphilis.

The cause of this phenomenon has not been fully explained. As it has been observed only when the antisheep system is being used, one is justified in seeking the source of this irregularity in the fluctuation of the amount of natural antisheep amboceptor in the serum of the patient during the treatment. A serologist should expect a certain degree of irregularity due to the use of an heterohemolytic system through variable and inconstant fluctuations of the natural hemolytic amboceptors in the patient's serum. By adding immune hemolytic amboceptors, he can produce experimentally conditions which will cause a positive serum to give a negative reaction. This result is always obtained when the reaction is not very strong.

In practice it has been repeatedly demonstrated that the antihuman hemolytic system of Noguchi reveals the syphilitic antibody where the Wassermann system fails on account of the presence of natural amboceptor. Any one who has used both methods adequately must have concluded that the Noguchi system gives a more delicate and constant result with the serum of the patient under treatment. Pedersen, who utilized the antihuman system of Nogu-

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chi, in following up the effects of treatment found a perfect harmony between the intensity of the reaction and the clinical course of the disease during the treatment.

Efforts have been made by some investigators to discover whether or not the sudden disappearance or weakening of the reaction is the direct effect of the mercury upon the syphilitic antibody. Their results are contradictory, as some claim to have obtained a negative reaction with a positive syphilitic serum by mixing it with soluble mercurial preparations in test tubes, while others observed no such effects.

We have made some experiments to ascertain what effects a soluble mercurial preparation will have on the syphilitic antibody *in vitro*. As mercurial preparation, we have chosen mercuric bichloride; and for the fixation test, the system of Noguchi.

The effect of bichloride of mercury was determined upon (1) red blood corpuscles, (2) complement, (3) antihuman amboceptor (rabbit), (4) syphilitic antigen (aceton-insoluble tissue lipoids of human liver), and (5) syphilitic antibody. The results obtained are given briefly in what follows.

Hemolytic Power of Bichloride of Mercury.—In a series of tubes, each containing one cubic centimeter of a 1 per cent. suspension of washed human corpuscles, varying amounts of bichloride of mercury in suitable concentrations were added and the mixtures were incubated for two hours at 37° C. The results are as follows:

Bichloride of mercury in grams.	
0.001	No hemolysis (precipitate, discoloration)
0.0003	30 per cent. hemolysis (precipitate)
0.0001	100 per cent. hemolysis (precipitate)
0.00003	100 per cent. hemolysis
0.00001	100 per cent. hemolysis
0.000003	100 per cent. hemolysis
0.0000025	100 per cent. hemolysis
0.000002	95 per cent. hemolysis
0.0000015	90 per cent. hemolysis
0.000001	No hemolysis
0.0000007	No hemolysis

The hemolytic power of bichloride of mercury was considerably reduced by mixing it with 0.05 cubic centimeter of guinea pig complement or with two units of the amboceptor serum on paper.

Bichloride of mercury in grams.	0.05 c.c. complement (incubated 1 hour before addition of corpuscles).	2 units amboceptor (paper) (incubated 1 hour before addition of corpuscles).
0.000005	100 per cent. hemolysis	100 per cent. hemolysis
0.000003	No hemolysis	100 per cent. hemolysis
0.0000025	No hemolysis	100 per cent. hemolysis
0.000002	No hemolysis	60 per cent. hemolysis
0.0000015	No hemolysis	No hemolysis
0.000001	No hemolysis	No hemolysis

Thus, the original strength, 0.0000025, for complete hemolysis became 0.000005, and the non-hemolytic quantity, 0.000001, became 0.000003 after the bichloride had been digested with 0.05 cubic centimeter of complement. It is evident, therefore, that 0.05 cubic centimeter of complement absorbed the differences during one hour's contact with the sublimate. The absolute quantity of bichloride of mercury absorbed by this amount of serum is about 0.000002 gram. The amboceptor serum interfered in a lesser degree.

The hemolytic power of bichloride of mercury was not noticeably diminished by the antigen, which was used in doses of 0.0003 gram.

Effects of Bichloride of Mercury upon Complement and Amboceptor.—In order to detect any possible anticomplementary property of bichloride of mercury, 0.005 cubic centimeter of guinea pig complement was mixed with varying quantities of bichloride of mercury and the mixtures were incubated at 37° C. for one hour. At the end of incubation the corpuscle suspension and two units of amboceptor were introduced, and the mixtures were again incubated at 37° C.

Bichloride of mercury in grams.	Complement 0.005 + Amboceptor (two units).	Complement 0.005 + No amboceptor (control).
0.0000025	Complete hemolysis	Complete hemolysis
0.0000015	Complete hemolysis	No hemolysis
0.000001	Complete hemolysis	No hemolysis
0.0000006	Complete hemolysis	No hemolysis
0.0000003	70 per cent. hemolysis	No hemolysis
control	70 per cent. hemolysis	No hemolysis

The experiment indicates that bichloride of mercury has no anticomplementary effect in the quantities used. On the contrary, the hemolysis was more complete and proceeded more rapidly in its

presence. This is due to the injurious effect of bichloride of mercury upon the corpuscles, thus reducing the corpuscular resistance to the hemolytic action brought about by the complement and amboceptor.

Effects of Bichloride of Mercury upon the Syphilitic Antigen and Antibody.—To determine the effect of bichloride of mercury upon the antigen, it was mixed with the latter and incubated for one hour at 37° C. before introducing the syphilitic serum and complement. After the incubation, the syphilitic serum and complement were added and incubated, as usual. Amboceptor and corpuscles were added and the whole was again incubated.

To determine the effect of bichloride of mercury upon the syphilitic antibody, it was mixed with the latter and incubated for one hour at 37° C., then, at the end of incubation, the complement and antigen were introduced, the remaining procedures being the same as usual.

The amount of complement employed was 0.05 cubic centimeter; that of amboceptor, two units; that of antigen, 0.0003 gram of aceton-insoluble lipoids; that of syphilitic serum, 0.02 cubic centimeter; and that of corpuscles, 0.1 cubic centimeter of a 10 per cent. suspension. The total volume of fluid in each tube equalled one cubic centimeter.

The foregoing experiments show that bichloride of mercury in the highest non-hemolytic quantity (0.0000025 gram) failed to remove, to a noticeable degree, the syphilitic antibody, and the reaction in two strongly positive sera remained unchanged. In two weaker sera there was a little more hemolysis than in the control tubes which contained no bichloride of mercury. This slight hemolysis may be explained, (1) by the removal of some of the antibodies by the bichloride, (2) by the injury inflicted by the bichloride upon the red blood corpuscles, rendering these more readily hemolyzable by the complement and amboceptor introduced afterward, or (3) by the combined action of (1) and (2). Of these possibilities, the second is the more probable, this assumption being supported by the fact that, in the controls in which no bichloride of mercury was present, and in which no hemolysis occurred at first, there was a trace of hemolysis after these had stood for six

TABLE I.

Experimental arrangement.	Effect of $HgCl_2$ upon syphilitic antigen. Antigen (0.0003 gram) + $HgCl_2$; incubation. Then, complement (0.05 c.c.) and syphilitic serum (0.02 c.c.); incubation. Then, corpuscular suspension and amboceptor; incubation.				Effect of $HgCl_2$ upon syphilitic antibody. Syphilitic serum (0.02 c.c.) + $HgCl_2$; incubation. Then, complement (0.05 c.c.) and antigen (0.0003 gram); incubation. Then, corpuscular suspension and amboceptor; incubation.			
Varieties of serum.	Secondary lues. Serum No. 1.	Secondary lues. Serum No. 2.	Tertiary lues. Serum No. 3.	Latent lues. Serum No. 4.	Serum No. 1.	Serum No. 2.	Serum No. 3.	Serum No .
$HgCl_2$(gram)								
0.0000025	No H.	No H.	Slight H.	Complete H.	No H.	No H.	Slight H.	Complete I .
0.000002	No H.	No H.	Trace H.	Almost complete H.	No H.	No H.	Trace H.	Complete H.
0.0000015	No H.	No H.	No H.	Much H.	No H.	No H.	No H.	Much H.
0.000001	No H.	No H.	No H.	Moderate H.	No H.	No H.	No H.	Moderate H.
0.0000006	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.
0.0000003	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.
control	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.

Absolute inhibition. (Secondary lues. Serum No. 1.)

Absolute inhibition. (Secondary lues. Serum No. 2.)

Trace H in 20 hours. (Tertiary lues. Serum No. 3.)

Trace H in 6 hours. (Latent lues. Serum No. 4.)

Trace H in 20 hours. (Serum No. 3.)

Trace H in 6 hours. (Serum No .)

H = hemolysis.

hours or longer, showing that the complement was not completely fixed. The same is true also in the experiments relating to the effect of sublimate upon the antigen. When working without proper controls (such as determine the effects of non-hemolytic doses of bichloride of mercury upon the complement-amboceptor hemolysis), one is apt to consider the above experiments as constituting evidence of the removal of the syphilitic antibody by the sublimate. It may be added here that the strongly positive sera (No. 1 and No. 2) contained only about two units of syphilitic antibody in 0.02 cubic centimeter. In test tube experiments it seems to us that the only way to determine the slightest possible effect of sublimate upon syphilitic antibody is to test the action of the highest non-hemolytic quantity of bichloride of mercury upon the smallest detectable number of antibody units. This was done in our present determination.

Whether the same results are obtainable with the antisheep hemolytic system is another problem. If the resistance of sheep corpuscles to the hemolytic effect of bichloride of mercury is greater, thus permitting the use of a larger amount of the mercurial salt, a more distinct effect might be secured. But the work of Satta and Donati shows it to be about the same. One might imagine that the hemolytic effect of the mercurial salt can be prevented by adding certain indifferent proteins to the mixture, but, unfortunately, according to our recent studies, the indifferent proteins can themselves saturate the fixing affinity of the mixture of syphilitic serum and antigen and render the positive reaction negative.

The test tube experiments do not, however, exclude the possibility that bichloride of mercury acts in the body upon the syphilitic antibody. In the body the sublimate circulates constantly and may remove the syphilitic antibody little by little. Besides, the rich protein content of the body fluids, especially the blood serum, protects the red blood corpuscles from the injurious effect of bichloride of mercury to a far greater degree than the conditions *in vitro* permit. The question is not adequately answered by means of test tube experiments. It is well to recall here that the administration of the powerful spirochæticide, dioxy-diamido-arsenobenzol,

of Ehrlich and Hata can lead to the disappearance of the syphilis reaction in a comparatively short period, yet this preparation contains no mercury. The disappearance of the reaction under the mercurial treatment may, therefore, be due, not to an influence on the antibody, but to a destruction of the spirochæte.

BARIUM SULPHATE ABSORPTION AND THE SERUM DIAGNOSIS OF SYPHILIS.*

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Negative serum reactions in untreated, well-developed cases of secondary syphilis have been sometimes encountered by those who employ the original method of Wassermann. According to Wechselmann, whose experiments were carried out under Wassermann, this is said to be due to the development of complementoid in the patient's serum during inactivation, and inactivation is essential for Wassermann's method. Wechelsmann states that the treatment of such specimens of syphilitic sera with barium sulphate removes the interfering complementoid and causes the treated sera to react positively. A later report by Lange contains a list of a large number of sera which either became positive or stronger after treatment with barium sulphate. According to Wechselmann and Lange, every specimen of serum sent for diagnosis must be treated with barium sulphate in order to insure a reliable result. Although the absorption of serum with barium sulphate is a simple enough process, yet before its routine adoption in the complement fixation test by the Wassermann method, further examination is demanded. Whatever may be the cause, the phenomenon itself is of interest. In our studies of this subject we employed the antihuman hemolytic system, and have considered the following questions:

1. The antibody content before and after barium sulphate absorption.

2. The effect of barium sulphate upon the complementary activity of guinea pig serum.

3. The effect of barium sulphate upon the antibody content of different syphilitic sera.

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4. The effect of the addition of normal serum upon the barium sulphate absorption of syphilitic antibody.

5. The fate of syphilitic antibodies after barium sulphate absorption.

6. The absorption of hemolytic amboceptors by barium sulphate.

1. *The Antibody Content before and after Barium Sulphate Absorption.*—In this series of experiments we employed unheated and inactivated syphilitic sera. The mixtures of the serum and barium sulphate were made in the proportions originally recommended by Wechselmann. The result shows that the absorption of unheated as well as inactivated serum renders the reaction somewhat stronger, especially with the inactivated sera. This experiment confirms the finding of Wechselmann with inactivated serum, but does not prove that it was the removal of the complementoid that made the reaction stronger. The barium sulphate removed certain serum constituents which interfere with the fixation of the complement. The increase in the intensity of reaction after the

TABLE I.

Absorption with $BaSO_4$ Increases the Number of Antibody Units.

Amounts of the dilution of the centrifugate used for the fixation test.	Unheated syphilitic serum.		The same serum after inactivation.	
	Syphilitic serum 0.1 c.c. + $BaSO_4$ (7%) 0.055 c.c.; incubation; centrifugalization. The centrifugate was made equal to 5 c.c. with salt solution and used for the fixation test.	Control without $BaSO_4$ treatment.	**Syphilitic serum (56° C.) 0.1 c.c. + $BaSO_4$ (7%) 0.055 c.c.; incubation; centrifugalization.** The centrifugate was made equal to 5 c.c. with salt solution and used for the fixation test.	Control without $BaSO_4$ treatment.
1.0	No hemolysis	No hemolysis	No hemolysis	No hemolysis
0.75	No hemolysis	No hemolysis	No hemolysis	No hemolysis
0.625	No hemolysis	No hemolysis	No hemolysis	Slight hemolysis
0.5	No hemolysis	No hemolysis	No hemolysis	Much hemolysis
0.4	No hemolysis	No hemolysis	Much hemolysis	Complete hemolysis
0.3	No hemolysis	No hemolysis	Complete hemolysis	Complete hemolysis
0.25	No hemolysis	No hemolysis	Complete hemolysis	Complete hemolysis
0.2	No hemolysis	No hemolysis	Complete hemolysis	Complete hemolysis
0.15	Trace hemolysis	Slight hemolysis	Complete hemolysis	Complete hemolysis
0.1	Moderate hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
0.075	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
0.05	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
	$\frac{5}{0.16}=30$ antibody units	$\frac{5}{0.175}=28.5$ antibody units	$\frac{5}{0.5}=10$ antibody units	$\frac{5}{0.75}=6.6$ antibody units

barium sulphate absorption is almost negligible with the unheated sera.

The results in table I show that a certain quantity of barium sulphate mixed with the syphilitic serum strengthens the reaction. It was of interest, therefore, to see whether barium sulphate in other proportions might not remove the syphilitic antibody. The results of experiments determining this point are recorded in table II.

TABLE II.

Removal of Syphilitic Antibody by Absorption with $BaSO_4$.

	Syphilitic serum No. 1.		Syphilitic serum No. 2.	
	0.002 c.c.	0.02 c.c.	0.003 c.c.	0.03 c.c.
$BaSO_4$ (7 per cent.)				
1.0	Complete H.	Complete H.	Complete H.	Complete H.
0.7	Complete H.	Complete H.	Complete H.	Complete H.
0.5	Complete H.	Complete H.	Complete H.	Complete H.
0.4	Complete H.	Complete H.	Complete H.	Complete H.
0.3	Complete H.	Much H.	Complete H.	Complete H.
0.25	Complete H.	No H.	Complete H.	Much H.
0.2	Complete H.	No H.	Complete H.	No H.
0.15	Complete H.	No H.	Complete H.	No H.
0.1	Complete H.	No H.	Complete H.	No H.
0.07	Complete H.	No H.	Complete H.	No H.
0.05	Complete H.	No H.	Complete H.	No H.
0.04	Complete H.	No H.	Complete H.	No H.
0.03	Much H.	No H.	Complete H.	No H.
0.025	No H.	No H.	Complete H.	No H.
0.02	No H.	No H.	Much H.	No H.
0.015	No H.	No H.	No H.	No H.
0.01	No H.	No H.	No H.	No H.
0.007	No H.	No H.	No H.	No H.
0.005	No H.	No H.	No H.	No H.
control	No H.	No H.	No H.	No H.
Amount of $BaSO_4$ (7 per cent.) required for complete absorption.	0.04 c.c.	0.4 c.c.	0.025 c.c.	0.3 c.c.

H = hemolysis.

Table II shows that the syphilitic antibody can be completely removed by absorption with a sufficiently large amount of barium sulphate. With a given serum the amounts of the antibodies removed by barium sulphate are approximately proportional to the amounts of barium sulphate employed. Thus, to remove ten times the number of antibody units, ten times the quantity of barium sulphate is required. On the other hand, we have noticed that the

absolute amount of the sera containing the antibodies bears no quantitative relationship to the amount of barium sulphate required for different sera. In the experiment (table II), a somewhat larger amount of barium sulphate is seen to be necessary to remove the antibodies from a somewhat smaller amount of the serum. The number of antibody units contained in 0.002 cubic centimeter of the first specimen was the same as that in 0.003 cubic centimeter of the second. From this fact it is clear that the absorption by barium sulphate is not directed in a selective manner to the antibodies.

2. *The Effect of Barium Sulphate upon the Complementary Activity of Guinea Pig Serum.*—Incidentally, it may be mentioned that barium sulphate has almost no anticomplementary action upon guinea pig serum. A slight antihemolytic effect has been noticed when one cubic centimeter of a 7 per cent. suspension of this salt was mixed with 0.05 cubic centimeter of the complement.

3. *The Effect of Barium Sulphate upon the Antibody Content of Different Syphilitic Sera.*—In this series of experiments we selected six samples of syphilitic sera, the antibody contents of which were very variable. The titers of these sera are recorded in table III, where other details of the experiments are also given.

The results were rather unexpected and reveal wide variations in the ease or difficulty with which the removal of the antibodies from different sera could be effected with barium sulphate absorption. At the foot of the table we have recorded figures for the amounts of barium sulphate necessary for removing one antibody unit from different sera and the relative quantities of the barium salt necessary to absorb a given unit volume of these six sera. This numerical expression aids us in seeing how each specimen behaved towards the barium sulphate absorption. The most difficult serum to fix, No. 1, is found to be nearly forty times less sensitive to the barium sulphate absorption than the most easily fixable specimen of serum, No. 2. Here again we obtain the evidence that the absorption by barium sulphate is not proportional to the amount of the serum. Nor is it parallel to the antibody unit, because the amount of each serum employed in this experiment represented about one unit. If the absorption were selectively directed toward the antibodies, the amount of barium sulphate necessary to remove

TABLE III.

Antibody Content of Different Sera Removed by $BaSO_4$.

	Determination of the quantities of $BaSO_4$ necessary to remove 1 unit of syphilitic antibody from different samples of positive sera.					
	Serum No. 1. Titer, 0.00125 c.c.	Serum No. 2. Titer, 0.00175 c.c.	Serum No. 3. Titer, 0.0025 c.c.	Serum No. 4. Titer, 0.0125 c.c.	Serum No. 5. Titer, 0.0125 c.c.	Serum No. 6. Titer, 0.025 c.c.
$BaSO_4$(7 %)						
0.5	Complete H.	Complete H.	Complete H.	Complete H.	Complete H.	Complete H.
0.4	Complete H.	Complete H.	Complete H.	Complete H.	Complete H.	Complete H.
0.3	Complete H.	Complete H.	Complete H.	Complete H.	Complete H.	Complete H.
0.2	Complete H.	Complete H.	Complete H.	No H.	Complete H.	Complete H.
0.1	No H.	Complete H.	Complete H.		Moderate H.	No H.
0.07		Complete H.	No H.		No H.	
0.05		Complete H.				
0.04		Complete H.				
0.03		Complete H.				
0.02		Complete H.				
0.015		Complete H.				
0.01		Complete H.				
0.007		Complete H.				
0.005		No H.				
control	No H.	No H.	No H.	No H.	No H.	No H.
$BaSO_4$ for removal of unit antibody	0.2 c.c.	0.007 c.c.	0.1 c.c.	0.3 c.c.	0.2 c.c.	0.2 c.c.
$BaSO_4$ for removal of 1 volume unit of serum	$\frac{0.2}{0.00125} = 160$	$\frac{0.007}{0.00175} = 4$	$\frac{0.1}{0.0025} = 40$	$\frac{0.3}{0.0125} = 24$	$\frac{0.2}{0.0125} = 16$	$\frac{0.2}{0.025} = 8$

H = hemolysis.

the antibodies would have been nearly the same for all specimens. Just what determines this variation in the fixability of human serum by barium sulphate was not investigated further.

In the next series of experiments we determined whether barium sulphate absorbs the anticomplementary substances of human serum as in the case of the syphilitic antibodies. For this purpose we selected some syphilitic sera which became anticomplementary upon standing for many days. The results are recorded in table IV.

The experiments in table IV demonstrate that barium sulphate removes both the anticomplementary substances and the syphilitic antibodies.

4. *The Effect of the Addition of Normal Serum Upon the Barium Sulphate Absorption of Syphilitic Antibody.*—We have already shown that barium sulphate removes the antibodies from a syphilitic

TABLE IV.

Removal of the Syphilitic Antibodies and Anticomplementary Substances with $BaSO_4$.

0.02 c.c. of each serum was mixed with 1 c.c. of $BaSO_4$ (7 per cent.) and incubated at 37°C. for 1 hour. The mixture was centrifugalized and the centrifugate was tested for the antibody in the usual way. The fixation test was made with 0.05 c.c. and 0.1 c.c. of guinea pig complement. Controls were also made with the untreated sera.

		Serum No. 1.		Serum No. 2.		Serum No. 3.		Serum No. 4.	
		No antigen.	Plus antigen.	No antigen.	Plus antigen.	No antigen.	Plus antigen	No antigen.	Plus antigen.
Serum treated with $BaSO_4$	Complement 0.05 c.c.	Complete H.	Complete H.	Complete H.	Complete H.	Complete H.	Complete H.	Complete H.	Complete H.
	Complement 0.1 c.c.	Complete H.	Complete H.	Complete H.	Complete H.	Complete H.	Complete H.	Complete H.	Complete H.
Untreated serum (control)	Complement 0.05 c.c.	No H.	No H.	No H.	No H.	Slight H.	No H.	No H.	No H.
	Complement 0.1 c.c.	Complete H.	Much H.	Complete H.	No H.	Complete H.	No H.	Complete H.	No H.

		Serum No. 5.		Serum No. 6.		Serum No. 7.	
		No antigen.	Plus antigen.	No antigen.	Plus antigen.	No antigen.	Plus antigen.
Serum treated with $BaSO_4$	Complement 0.05 c.c.	Complete H.	Complete H.	Complete H.	Complete H.	Complete H.	Complete H.
	Complement 0.1 c.c.	Complete H.	Complete H.	Complete H.	Complete H.	Complete H.	Complete H.
Untreated serum (control)	Complement 0.05 c.c.	Slight H.	No H.	Slight H.	No H.	Slight H.	No H.
	Complement 0.1 c.c.	Complete H.	No H.	Complete H.	No H.	Complete H.	No H.

serum in almost direct proportion to the amount of this salt used for absorption. We have also stated that the treatment of some syphilitic sera with a *comparatively small amount* of barium sulphate removes certain serum constituents and renders the reaction somewhat stronger than in the untreated serum. We have also shown elsewhere that when the syphilitic antibody is associated with comparatively large amounts of normal serum constituents, the fixation reaction is proportionately less sensitive. Reversing this consideration, we derive the conclusion that by removing the normal serum constituents as much as possible we can make the reaction more distinct; in other words, we might be able to purify the syphilitic antibodies. Such a purification is possible, especially when the other interfering serum constituents possess a greater affinity than the antibodies for barium sulphate. It is also possible to purify the syphilitic antibodies to a certain extent, even when the affinities of the antibodies and other serum constituents for barium sulphate absorption are the same, provided the amount of the serum constituents is greater than that of the antibodies. This is not possible, however, when the amounts of the indifferent serum constituents and the antibodies are in the reverse proportion, because here we would remove more antibodies than interfering serum constituents. It is, therefore, necessary to determine experimentally whether the non-syphilitic serum possesses the same affinity for barium sulphate as the antibodies of syphilitic serum do. We have accordingly estimated the number of the antibody units after barium sulphate absorption in syphilitic serum, syphilitic serum plus an equal amount of normal serum, and syphilitic serum plus four times the quantity of normal serum. If the normal serum possesses the same affinity as the syphilitic antibodies, its presence must interfere with the removal of the latter by a given amount of barium sulphate.

The protocol given in table V shows that the addition of one part of normal serum to one part of syphilitic serum reduced the removal of the antibodies to one-half of that obtained when no normal serum was added, and the addition of four parts of the normal serum to one part of syphilitic serum reduced the removal to about one-fifth. This indicated undoubtedly that the normal serum em-

TABLE V.

Interference by Normal Serum with the $BaSO_4$ Absorption of Syphilitic Antibody.

Amounts of diluted centrifugate used in the fixation test with antigen.	Series 1. Syphilitic serum and $BaSO_4$. **Syphilitic serum (1:10) 1.2 c.c. + $BaSO_4$ (7%) 0.6 c.c.; incubation at 37° C., 1 hour; centrifugalization.** The centrifugate is made equal to 25 c.c. with salt solution.	Series 2. Syphilitic serum and normal serum, equal parts, and $BaSO_4$. **Syphilitic serum (1:10) 1.2 c.c. + normal serum (1:10) 1.2 c.c. + $BaSO_4$ (7%) 0.6 c c.; incubation; centrifugalization.** The centrifugate is made equal to 25 c.c. with salt solution.	Series 3. Syphilitic serum with 4 parts of normal serum, and $BaSO_4$. **Syphilitic serum (1:10) 1.2 c.c. + normal serum (undiluted) 0.5 c.c. + $BaSO_4$ (7%) 0.6 c.c.; incubation; centrifugalization.** The centrifugate is made equal to 25 c.c. with salt solution.	Series 4. Control: syphilitic serum alone without $BaSO_4$. **Syphilitic serum (1:10) 1.2 c.c. + salt solution 0.6 c.c.; incubation; centrifugalization.** The fluid is made equal to 25 c.c. with salt solution.
1.0	No hemolysis.	No hemolysis.	No hemolysis.	No hemolysis.
0.7	Much hemolysis.	No hemolysis.	No hemolysis.	No hemolysis.
0.5	Complete hemolysis.	No hemolysis.	No hemolysis.	No hemolysis.
0.4	Complete hemolysis.	No hemolysis.	No hemolysis.	No hemolysis.
0.3	Complete hemolysis.	Almost complete hemolysis.	No hemolysis.	No hemolysis.
0.25	Complete hemolysis.	Complete hemolysis.	Trace hemolysis.	No hemolysis.
0.2		Complete hemolysis.	Complete hemolysis.	Much hemolysis.
0.15		Complete hemolysis.	Complete hemolysis.	Complete hemolysis.
0.1		Complete hemolysis.	Complete hemolysis.	Complete hemolysis.
0.07			Complete hemolysis.	Complete hemolysis.
0.05				Complete hemolysis.
0.04				Complete hemolysis.
0.03				Complete hemolysis.
	$\frac{25}{1}$ =25 units; removed 75 units;	$\frac{25}{0.4}$ =62.5 units; removed 37.5 units;	$\frac{25}{0.3}$ =83.3 units; removed 16.6 units;	$\frac{25}{0.25}$ =100 units; original content.

ployed, possessed the same affinity as the syphilitic antibodies for barium sulphate. In another experiment we found that the number of demonstrable antibodies in a mixture of normal and syphilitic sera, in the proportions of 1 to 1 and 1 to 4, was considerably smaller when barium sulphate was not used than when it was used. As we have shown in another communication, when normal serum[1] is added to a mixture containing syphilitic antigen and antibody, it interferes with the fixation of complement subsequently to be added, by uniting with the molecules which under the usual conditions are capable of fixing complement.

It would manifestly be unsafe to conclude from these experiments that all normal sera behave in the same way. On the con-

[1] In fixation experiments, white of egg has a strong interfering property which can be removed to a large extent by absorption with barium sulphate.

trary, we already know that there are many human sera which fix with great difficulty.

5. *The Fate of Syphilitic Antibodies after Barium Sulphate Absorption.*—We have endeavored to trace the antibodies which disappear during the process of barium sulphate absorption. The results are given in table VI.

TABLE VI.

Fate of the Syphilitic Antibody after $BaSO_4$ Absorption.

		Syphilitic serum (1:20) 0.6 c.c. + $BaSO_4$(7%) 0.6 c.c.; incubation; centrifugalization.		Control: no $BaSO_4$ absorption; syphilitic serum (1:20) 0.6 c.c. in 4 c.c. salt solution.
		Sediment resuspended in 4 c.c. salt solution, and used for fixation test.	Centrifugate was made equal to 4 c.c. with salt solution and used for fixation test.	
		1	2	3
Quantities of: (1) sediment suspension, (2) $BaSO_4$ treated serum, (3) untreated serum (control) for fixation test. Complement, 0.05 c.c. The rest of the procedure was as usual.	1.0		No H.	
	0.8		Moderate H.	
	0.6	No H.	Complete H.	No H.
	0.5	No H.	Complete H.	No H.
	0.4	No H.	Complete H.	No H.
	0.3	Complete H.		No H.
	0.25	Complete H.		No H.
	0.2	Complete H.		Much H.
	0.1	Complete H.		Complete H.
Number of antibody units contained in the total 4 c.c. of each series.		10 units.	4 units.	16 units.

The experiments given in table VI show (1) that the syphilitic antibodies are removed by barium sulphate (to which they seem to adhere), and (2) that the antibodies thus absorbed retain their activity.

6. *The Absorption of Hemolytic Amboceptors by Barium Sulphate.*—Several experiments were performed with antihuman amboceptor (rabbit), and the results show that the amboceptpor is quite readily removed from the solution by barium sulphate absorption. The barium sulphate treated with the amboceptor solution retains the amboceptor and causes hemolysis when mixed with human corpuscles and guinea pig complement. Although we have not made similar absorption tests with antisheep amboceptor, it is probable that this acts in the same way.

CONCLUSIONS.

The so-called syphilitic antibodies can be removed from a serum by means of absorption with barium sulphate. The removal is due either to an adsorption or a mechanical absorption. The activity of the syphilitic antibodies is thereby unimpaired. The readiness with which the absorption is accomplished with barium sulphate varies considerably with different syphilitic sera. That barium sulphate exerts the same absorbing effect upon non-syphilitic serum components is made evident by the interfering property which the latter manifest in the absorption experiment of the syphilitic antibodies. The selective removal of the serum components, other than the syphilitic antibodies, by means of barium sulphate absorption is, therefore, impossible.

On the other hand, a partial removal of these components, with but little removal of the syphilitic antibodies, may be effected when the content of a given serum is poor in syphilitic antibodies and comparatively rich in the indifferent serum components. But this is impossible if the conditions are reversed. The main reasons why some negative syphilitic sera may be so modified by the barium sulphate treatment as to give positive reactions, are explained below, but these apply only to those methods in which inactivated serum is employed. The inactivation reduces the antibody content to about one-fourth to one-fifth of the original. When the serum is very rich in antibodies, this does not affect the result of the fixation test. But when the amount of the antibodies is small, the process of inactivation creates conditions quite unexpected. It may produce such a condition that a given amount of the serum contains, after inactivation, only one or two antibody units, while the other serum components remain undiminished. Here one must not lose sight of the vital fact that these apparently indifferent serum constituents are not at all indifferent in the fixation processes. They may possess affinities which are similar to those of complement for the fixing combination of syphilitic serum and antigen. Speaking quantitatively, one unit of the syphilitic antibodies plus antigen will fix 0.1 cubic centimeter of guinea pig complement, but this unit can also be saturated and blocked by nearly the same amount of the seemingly indifferent

serum component of the serum to be tested. Moreover, the regular amount of inactivated serum used in the Wassermann system is 0.2 cubic centimeter, a quantity sufficient to saturate two units of the fixing combination. Fortunately, this *self-saturation* of the syphilitic antibody-antigen combination by the other serum components is not constant in occurrence, owing to the wide variations of the fixability of the serum components of man. Here the benefit of Wechselmann's procedure becomes obvious. By removing a surplus of the fixable indifferent serum components by means of barium sulphate, the serum is made to react positively, or more strongly than before the treatment with barium sulphate. This masking of the positive reaction through the self-saturation is liable to occur in any system in which inactivated serum is recommended.

Another equally important factor in masking the positive reaction in a serum in which the antibodies are poor, is the presence in considerable amount of natural antisheep amboceptor in human serum. It is a plain and simple fact that an excess of hemolytic amboceptor renders a positive reaction feeble or completely negative. As we have shown in our present investigation, a hemolytic amboceptor can be removed from the serum by means of absorption with barium sulphate. Thus it is easy to understand why Wechselmann found that barium sulphate absorption improves the reaction in the original Wassermann system.

The treatment of syphilitic serum with this salt can have a twofold benefit in the case of the original method of Wassermann; namely, the removal of certain interfering serum components and the removal or diminishing of the natural antisheep amboceptor present in the syphilitic serum.

In the method of Noguchi, there is no necessity for applying the barium sulphate absorption. Noguchi recommends the use of unheated serum, hence the absolute amount of the serum employed is only one-half of the absolute amount of complement. Eventually an old serum may be anticomplementary and need inactivation, but if the result is doubtful in this instance, a fresh serum from the same patient may be secured and subjected to reëxamination. In this method there is no danger of introducing an amount of hemolytic amboceptor which is both unknown and uncontrollable, for the

reason that human serum is usually devoid of isolysin (antihuman hemolytic amboceptor), and, if the latter is present, it never reaches the strength which shows any effect upon the hemolytic system employed.

We conclude, therefore, that the barium sulphate absorption is to be recommended for the original method of Wassermann under certain conditions, but that it is unnecessary for the antihuman hemolytic system of Noguchi.

EFFECTS OF MECHANICAL AGITATION AND OF TEMPERATURE UPON COMPLEMENT.*

By HIDEYO NOGUCHI and J. BRONFENBRENNER.

(*From the Laboratories of the Rockefeller Institute for Medical Research, New York.*)

In making the fixation tests, the mixtures employed must be frequently shaken, and moderately high temperatures are used. It is of importance, therefore, to know whether these procedures may affect the complement in any way. In order to answer this question the following experiments were undertaken.

THE EFFECTS OF MECHANICAL AGITATION.

The shaking of the serum was made at two different temperatures, at 10° C. and at 37° C. The oscillations were about forty per minute. The serum was sealed in a sterile glass tube without a cork, and the tube containing the serum was placed in a horizontal position in a shaking apparatus. The tube had space enough inside to allow the serum to undergo strong agitation. The titration was made at different periods. At the same time, other portions of the same serum were allowed to stand near the shaken tubes under experiment, and they were examined as controls.

The hemolytic experiments for titration of the complementary activity were made with two different amboceptors, one from an immunized rabbit and the other from an immunized goat. As usual, the amount of corpuscles was 0.1 cubic centimeter of a 10 per cent. suspension of washed human erythrocytes. The total volume was one cubic centimeter.

The foregoing experiments show that the complement of guinea pig serum is considerably injured by continuous shaking at 37° C. Within one hour the reduction was trifling, within three hours the strength of the shaken serum was only one-fourth to one-fifth of

* Received for publication, September 21, 1910.

TABLE I.

Shaking at 37° C.

Varieties of amboceptor.	Guinea pig serum.	1 hour at 37° C. Hemolysis.			3 hours at 37° C. Hemolysis.			6 hours at 37° C. Hemolysis.				
		100 per cent.	50 per cent.	25 per cent.	100 per cent.	50 per cent.	25 per cent.	100 per cent.	50 per cent.	25 per cent.	10 per cent.	0 per cent.
With antihuman amboceptor derived from immune rabbit.	No. 1 Shaken ..	0.0275	0.0125	0.008	0.1	0.04	0.02	—*	—*	0.15	0.005	0.03
	Unshaken	0.02	0.01	0.007				0.05	0.02	0.01	0.003	0.0015
	No. 2 Shaken ..	0.025	0.0125	0.007	0.1	0.04	0.02	—*	—*	0.145	0.05	0.03
	Unshaken	0.02	0.01	0.005				0.05	0.022	0.01	0.003	0.0015
	No. 3 Shaken ..	0.025	0.0125	0.007	0.1	0.04	0.02	—*	—*	—*	0.1	0.05
	Unshaken	0.02	0.01	0.005				0.04	0.02	0.01	0.003	0.0015
With antihuman amboceptor derived from immune goat.	No. 1 Shaken ..	0.015	0.007	0.004	0.06	0.03	0.02	—*	—*	0.15	0.05	0.03
	Unshaken	0.015	0.007	0.003				0.04	0.02	0.007	0.002	0.001
	No. 2 Shaken ..	0.015	0.007	0.004	0.06	0.03	0.02	—*	—*	0.15	0.05	0.03
	Unshaken	0.015	0.007	0.0035				0.03	0.015	0.006	0.002	0.001
	No. 3 Shaken ..	0.02	0.01	0.006	0.06	0.03	0.02	—*	—*	0.175	0.07	0.04
	Unshaken	0.02	0.01	0.005				0.03	0.015	0.007	0.0025	0.0015

*In quantities of .2 c.c., hemolysis did not reach the amount indicated.

TABLE II.

Shaking at 10° C.

Varieties of amboceptor.	Guinea pig serum.	1 hour at 10° C. Hemolysis.			24 hours at 10° C. Hemolysis.		
		100 per cent.	50 per cent.	25 per cent.	100 per cent.	50 per cent.	25 per cent.
With antihuman amboceptor derived from immune rabbit.	No. 1 Shaken.....	0.025	0.0125	0.006	0.05	0.025	0.0125
	Unshaken ...	0.02	0.01	0.005	0.04	0.0175	0.01
	No. 2 Shaken.....	0.022	0.0125	0.006	0.05	0.025	0.0125
	Unshaken ...	0.02	0.01	0.005	0.04	0.0175	0.01
	No. 3 Shaken.....	0.02	0.01	0.006	0.05	0.025	0.0125
	Unshaken ...	0.02	0.01	0.005	0.04	0.0175	0.008
With antihuman amboceptor derived from immune goat.	No. 1 Shaken.....	0.015	0.007		0.05	0.02	0.01
	Unshaken ...	0.015	0.007		0.03	0.01	0.005
	No. 2 Shaken.....	0.015	0.007		0.05	0.0175	0.0075
	Unshaken ...	0.015	0.007		0.03	0.0125	0.006
	No. 3 Shaken.....	0.015	0.007		0.05	0.0175	0.0075
	Unshaken ...	0.015	0.007		0.03	0.013	0.005

that of the fresh serum, and after six hours it was only one-fifteenth of that of the fresh serum. During the same periods, the unshaken serum also showed deterioration, and had after six hours only two-fifths of its original strength.

The effect of shaking was, however, much less injurious at 10°

C. Even after constant shaking at this temperature for twenty-four hours, the destruction of complement was not marked. The strength was reduced to a little less than half of what it was originally. The control had also deteriorated to half of its original power.

From this it appears that mechanical agitation at a low temperature has only a slightly destructive effect upon guinea pig complement, while at a higher temperature the complement is destroyed very rapidly, especially after the lapse of a few hours. In this last respect the destruction of complement differs markedly from that of certain ferments described by Meltzer and Shaklee.[1]

THE EFFECTS OF TEMPERATURE.

The effect of another physical force, heat, upon guinea pig complement has been studied by numerous investigators since the time of Buchner, and it is generally accepted that complement kept at 55° C. is destroyed in about thirty minutes. Ehrlich and Morgenroth showed, however, that goat complement resists this temperature, while one of us met with complements in cold-blooded animals which were destroyed, to a considerable extent, at 45° C. Zeissler found that the complement in human serum may still be active after heating the serum to 60° C. for one hour or longer.

Our study was made with six different samples of guinea pig serum. Each sample was divided into four parts, giving us four series. One of these was kept at room temperature as a control. The other three were heated for thirty minutes in a water bath at 45°, 50°, and 55° C. respectively.

Our reason for employing two different amboceptors was that we thought in this way we might be able to discover two different complements specific for each amboceptor, and that one might be destroyed while the other remained comparatively intact. But, as may be seen from the protocol, upon heating the sera at gradually increasing temperatures, the complementary activity for both amboceptors decreased progressively and equally. It was also quite unexpected to find that the complementary property was not completely lost at 55° C. in thirty minutes. It was, however, so weak-

[1] *Proc. Soc. Exper. Biol. and Med.*, 1908–9, vi, 103.

TABLE III.

Varieties of amboceptor.	Guinea pig serum.	Fresh serum. Hemolysis.				45° C.—30 minutes. Hemolysis.			50° C.—30 minutes. Hemolysis.				55° C.—30 minutes. Hemolysis.			
		50 per cent.	25 per cent.	15 per cent.	0 per cent.	50 per cent.	25 per cent.	0 per cent.	50 per cent.	25 per cent.	15 per cent.	0 per cent.	50 per cent.	25 per cent.	15 per cent.	0 per cent.
Amboceptor (rabbit)	No. 7	0.02	0.01	0.007	0.0025	0.04		0.02			0.1	0.05			0.25	0.075
	No. 8	0.02	0.01	0.007	0.002	0.025	0.015	0.01			0.1	0.05			0.2	0.075
	No. 9	0.02	0.01	0.007	0.002	0.025		0.0075			0.1	0.05			0.3	0.075
	No. 10	0.02	0.01	0.007	0.002	0.0275		0.01			0.1	0.035			0.15	0.075
	No. 11	0.015	0.007	0.004	0.002	0.02	0.015	0.01			0.1	0.04			0.15	0.075
	No. 12	0.0175	0.008	0.004	0.002	0.02	0.01	0.005			0.1	0.05			0.2	0.075
Amboceptor (goat)	No. 7	0.0125	0.005	0.0035	0.002	0.0125	0.007		0.15		0.075	0.02			0.25	0.05
	No. 8	0.015	0.0075	0.004	0.0015	0.015	0.0075			0.1		0.04			0.15	0.05
	No. 9	0.015	0.0075	0.004	0.0015	0.105	0.0075				0.1	0.05			0.15	0.04
	No. 10	0.01	0.005	0.003	0.0015	0.01	0.005				0.1	0.03			0.15	0.03
	No. 11	0.015	0.0075	0.004	0.0015	0.015	0.0075				0.1	0.04			0.15	0.05
	No. 12	0.015	0.0075	0.004	0.0015	0.015	0.0075				0.1	0.03			0.15	0.04

ened that to produce the same hemolytic effect as the original, at least thirty to forty times as large a quantity was required. The serum heated for thirty minutes at 50° C. had about one-fifteenth of the original strength, and at 45° C. it decreased to about one-half to one-third of the original. The reduction of the activity of the serum heated to 45° C. was scarcely detectable with goat amboceptor. But the destruction of complement for this and for rabbit amboceptor was equally marked when the serum was exposed to 50° and 55° C.

In the following experiments we determined the rate of destruction of this complement after various lengths of time, at the temperature of 50° C.

The protocol given above is of some interest. It will be noticed that the rate of destruction at 50° C. is not proportional to the length of time of exposure. During the first five minutes it was reduced to one-half of its original strength, but in the second five minute period the reduction was far less rapid. In the third and fourth five-minute periods, the reduction was again quite marked, while between the fourth and sixth five minute periods, the velocity of reduction was decreased. The periodic acceleration of physical force is by no means unusual. Certain ferments undergo a periodic destruction at higher temperatures.

TABLE IV.

	Guinea pig serum.	With rabbit amboceptor. Hemolysis.					With goat amboceptor. Hemolysis.				
		100 per cent.	50 per cent.	25 per cent.	10 per cent.	0 per cent.	100 per cent.	50 per cent.	25 per cent.	10 per cent.	0 per cent.
Unheated serum (original titers)	No. 1	0.022	0.01	0.006			0.0275	0.015	0.0075	0.0045	
	No. 2	0.02	0.01	0.007			0.0275	0.015	0.0075	0.0045	
Serum heated. 5 minutes at 50°C.	No. 1	0.0375	0.02	0.0125	0.006	0.0035	0.075	0.0225	0.015		0.005
	No. 2	0.0375	0.0175	0.0125	0.007	0.005	0.05	0.0225	0.015		0.005
10 minutes at 50°C.	No. 1	0.05	0.025	0.015	0.01	0.007	0.1	0.04	0.025	0.015	0.01
	No. 2	0.04	0.022	0.015	0.012	0.01	0.075	0.035	0.025	0.015	0.01
15 minutes at 50°C.	No. 1	0.1	0.05	0.03	0.025	0.015	0.15	0.075	0.04	0.022	0.015
	No. 2	0.08	0.04	0.025	0.015	0.01	0.15	0.075	0.035	0.022	0.015
20 minutes at 50°C.	No. 1	0.22	0.1	0.05	0.035	0.025		0.175	0.1	0.05	0.03
	No. 2	0.18	0.075	0.04	0.03	0.02		0.15	0.075	0.045	0.02
30 minutes at 50°C.	No. 1		0.22	0.1	0.05	0.025			0.125	0.075	0.045
	No. 2		0.2	0.1	0.04	0.025			0.125	0.075	0.05

CONCLUSIONS.

1. Under certain conditions, mechanical agitation destroys the complementary activity of guinea pig serum. It is most injurious when carried out constantly at a temperature of 37° C., but it is extremely insignificant at 10° C. After the first few hours at 37° C., the destruction of complement proceeded much more rapidly, and after six hours it was almost complete. On the other hand, within one hour shaking had almost no destructive effect on complement, even at 37° C. From this we may conclude that the several shakings which are necessary for fixation experiments during incubation do not modify perceptibly the outcome of the reactions.

2. The rate of destruction of the complement of guinea pig serum at temperatures above 45° C. is progressively greater as it approaches 55° C., at which temperature the activity is reduced in thirty minutes to one-thirtieth to one-fortieth of the original strength of the unheated serum; but it is not completely destroyed, as is commonly assumed.

The velocity of destruction of guinea pig complement when exposed to 55° C. for various lengths of time is found to be quite irregular, and not proportional to the length of time. This irregularity, however, presents a certain rhythm, a period of greater destruction alternating with one of less destruction.

MORPHOLOGICAL CHANGES OBSERVED IN A MOUSE CARCINOMA IN THE COURSE OF LONG-CONTINUED TRANSPLANTATION, AND THE INFLUENCE OF AN EXPERIMENTALLY PRODUCED DECREASE IN THE GROWTH-ENERGY OF THE TUMORS UPON THEIR MORPHOLOGICAL CHARACTER.*

By ROBERT KILDUFFE, 3d.

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Through the graded action of physical and chemical agencies upon the tumor material before inoculation, it is possible experimentally to decrease the energy of tumor growth, as was shown by Leo Loeb in his investigations in serial transplantations of thyroid sarcomas of rats.[1]

In a detailed investigation, a report of which will appear elsewhere, E. P. Corson White and Leo Loeb have studied the decrease in growth-energy produced by similar agencies in the carcinoma of white mice. It was of interest to investigate how far the morphological changes in such tumors corresponded to the experimentally produced variations in the energy of growth.

During the past year, therefore, a systematic microscopic study has been made of the different generations of a carcinoma transplanted into white mice. The original tumor had developed spontaneously in a mouse which Dr. Loeb obtained from Granby, Mass. The sections studied covered a period of experimentation with the tumor extending from March, 1907, to June, 1910, and comprised all generations produced within that time.

The sections were divided into four groups according to the history of the tumor.

(*a*) Sections of the original tumor transplanted without experimental interference or modification, "the unheated series."

* Received for publication, October 17, 1910.

[1] *Virchows Arch.*, 1903, clxxii, 345.

(*b*) Sections of the tumors which had been heated to 44° C. for varying periods of time before inoculation through successive generations, "the long-heated series."

(*c*) Sections of the tumors which had been heated before inoculation for varying periods of time as in (*b*) but through a shorter number of generations, "the short-heated series."

(*d*). Sections of tumors, of which some generations had been heated for varying lengths of time, and other generations had not been heated, "the mixed series."

The object of this investigation was to determine what changes in the morphological character, if any, took place as a result of the various manipulations to which this tumor had been subjected before inoculation.

The average interval separating the inoculation of the tumor and its removal for histological examination was approximately thirty days, although some tumors were removed as early as seven days, and some as late as fifty-four days after inoculation.

The tumor in its original state presented a distinctive picture. There was a more or less extensive central area of necrosis surrounded by living tumor tissue, which consisted of densely packed epithelial cells arranged in alveoli of rather large size, these alveoli being separated by thin strands of connective tissue. Here and there were seen smaller alveoli separated from the larger groups by connective tissue strands. These smaller alveoli presented very few indications of lumina. When present, however, these were seen to be formed by included lymph spaces or by sections of small blood-vessels. To distinguish them from true gland-like structures we shall designate these as "pseudo-acini."

As the center of a conglomeration of alveoli became necrotic, the outline of the central necrotic plug generally blocked out the surrounding masses of living tumor cells into long spiral formations. Such formations are hereafter spoken of as "gyrate formations." Where the gyrate formations predominated, the tumor was loose in texture.

Quite often the gyrate formations were seen in intimate relation to a blood vessel, being separated from it only by the wall of the vessel; on the other side of the gyrate formations necrotic

plugs were always present. There was little connective tissue to be seen in or around the gyrate formations. Mitoses were frequent, especially in tumors of rapid growth. Infiltration into the surrounding tissues was present in many, but not in all instances, and was never very marked. This, then, was the picture presented in the early generations of the tumor not subjected to experimental manipulations.

The first morphological change to be noted in the unheated series was the disappearance of the gyrate formations. These began to lessen numerically from the sixth to the eighth generation, disappearing in the tenth generation. This disappearance was due to the increase in the amount of connective tissue at the margin of a gyrate structure sending in smaller strands which separated it into smaller alveoli, so that what would have been a gyrate formation became, through the ingrowth of connective strands, a group of smaller alveoli. The disappearance of the gyrate formations was not absolute but depended upon the amount of connective tissue present, which was subject to wide variations; indeed, indications of gyrate formations, and even typical ones, are occasionally found in much later generations, *e. g.,* as late as the twenty-fifth generation, or even in tumors obtained quite recently.

In tumors grown in an individual mouse over a longer period of time, there was sometimes an increase in the amount of connective tissue, as a result of which such tumors tended toward the formation of smaller alveoli. While this increase in the amount of connective tissue is such that the older tumors generally contain slightly more connective tissue than the younger growths, it bears no quantitative relation to the length of time during which the tumor has grown in that individual mouse; but on the whole, the amount of connective tissue is subject to wide and sudden variations, a generation with much connective tissue being sometimes immediately succeeded by one in which the connective tissue was present in a small amount.

The amount of connective tissue is more or less independent of rapid or slow growth, heating, or experimental conditions of inoculation. The heating of the tumor material previous to the inoculation does not produce any definite increase in the amount

of connective tissue in the tumor that develops from this implantation.

Generally the tumors showed an alveolar structure, variations being produced through differences in the size, shape, and arrangement of the alveoli.

In no case were true acini found. Especially in the periphery of the tumors, where much connective tissue invaded the tumor parenchyma, apparent acini were occasionally observed, which, however, on closer investigation proved to differ from the grandular acini. We may call such structures pseudo-acini.

They were produced in one of the following ways:

1. By the breaking down of central cells. In this case, the lumen is occluded by cell debris, leaving a peripheral ring of tumor cells more or less bizarre in form. The same process produces a gyrate formation if the necrosis takes place in the center of large alveoli and if the necrosis breaks through the ring of tumor tissue at one or more points.

2. By the inclusion of blood spaces or small vessels in tumor alveoli.

3. By the inclusion of lymph spaces.

Pseudo-acini due to the second and third of the preceding ways were more frequently seen than those due to the first, and structures due to the third were the most frequent of all.

There was very little difference in the morphological character of slow and fast growing tumors.

Mitoses were present in all sections, varying somewhat in number but apparently without marked difference in sections of heated, unheated, or retrogressing tumors.

Infiltration of the surrounding tissues by the tumor cells was not constantly seen, and although there were sections showing tumor infiltration into muscle, fat, and areolar tissue, the infiltration was never very marked in any case.

The only marked effect of heating consisted in a retardation of growth. This effect varied generally with the duration of the heating.

The amount of necrosis, while independent of any law, varied considerably. It was perhaps greatest in those sections which were

obtained from tumors that had been heated the longest, but was often almost as extensive in unheated specimens.

We may conclude from the examination of these specimens that this tumor was but slightly, if at all, affected morphologically by the experimental manipulations (heating, etc.) to which it was subjected, for no permanent morphological change was produced through the influence of the physically injurious conditions to which it was exposed before inoculation. The partial disappearance of the gyrate formations which we described was evidently independent of the previous heating, and merely the result of successive transplantation. We wish to emphasize especially the fact that the experimentally produced decrease in the energy of growth of these tumors did not change the alveolar character to an acinous one. While structures which we designate as pseudo-acini were present, no true acini were found.

A return to an adenomatous structure, which Apolant[2] described as a result of weakening tumor growth through transplantation into resistant animals, was not observed after a direct weakening of the growth through the influence of physical agencies.

In conclusion I wish to express my thanks to Dr. Leo Loeb, at whose suggestion this work was undertaken.

[2] *München. med. Wchnschr.*, 1907, liv, 1720.

THE RELATIONS OF EMBRYONIC TISSUE AND TUMOR IN MIXED GRAFTS.*

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Plates X–XIV.

It has been often pointed out that the theory which gives to tumors an origin from misplaced embryonic tissue deals only with formal genesis, not with causal. As Bashford says, "Tumor disobeys the developmental laws regulating embryonic growth." Transplanted embryonic tissue will proliferate for a time, and may give rise to a teratoma that endures with the life of the host; but it shows a tendency to differentiate, and its growth is of limited nature—two characters which sharply distinguish it from tumor. A causal element which would explain its transformation into the latter is as much lacking as in the case of normal adult tissue.

Various experimental attempts have been made to stimulate implanted embryonic tissue to malignant growth. Neuhäuser[1] implanted organs at nearly the same site in adults that they had occupied in the fetus, and he obtained by this method, in one instance, a growth much like a true neoplasm. His results are still unconfirmed. Petrow[2] and Askanazy[3] attempted without success to stimulate with Scarlach R. grafts of embryonic tissue. Askanazy employed also four per cent. ether water and obtained by this means an excellent growth, but no true neoplasm. Alessandri[4] resorted to various physical and chemical methods without better

* Reported in the *Proc. Soc. Exper. Biol. and Med.*, 1910, vii, 73. Received for publication, November 4, 1910.

[1] Neuhäuser, H., *Deutsch. med. Wchnschr.*, 1909, xxxv, 332.

[2] Petrow, N. N., cited by G. Herxheimer and F. Reinke, *Ergebn. d. allg. Path.*, 1909, xiii, 405.

[3] Askanazy, M., *Rev. méd. de la Suisse romande*, 1909, xxix, 751.

[4] Alessandri, cited by G. Fichera, *Arch. de méd. exper. et d'anat. path.*, 1909, xxxi, 617.

result. And lastly, Shattock, Seligmann and Dudgeon,[5] assuming some inhibitive substance to be responsible for normal growth-cessation, have repeatedly injected embryonic tissue into the same individual, hoping thus to neutralize the inhibiting agent, after which unlimited growth might be expected to occur; but their attempts were not successful.

My own experiments to be described in this paper, though fruitful in other ways, must be classed with these as constituting an unsuccessful effort to stimulate embryonic tissue to malignancy. They were suggested by an instance of sarcomatous change in the stroma of a transplantable carcinoma, such as was first described by Ehrlich and Apolant,[6] and Loeb.[7] The evidence in these cases for a direct stimulation of normal tissue to malignancy is strong, and it has been rendered stronger by Russell's[8] demonstration that the length of time during which the carcinoma and stroma are associated is important in determining the malignant change. Acting on the idea that in this association embryonic tissue might prove more susceptible than adult, I have mixed it with tumor and implanted the two.[9] Nodules were thus obtained in which both had grown, sometimes intimately, but in the embryonic cells no tendency to a neoplastic change was noted. Perhaps the results would have been different had there been available a carcinoma with a tendency to cause a malignant change in its stroma. These growths are rare. For two experiments, however, a sarcoma of Ehrlich's that had arisen from the stroma of a carcinoma was employed.

The principal facts ascertained on examination of the nodules concern the conditions under which tumor and embryonic tissue can live side by side. The behavior of every tumor in its host, whether that be a rat or a man, gives data concerning a similar relationship between normal adult tissue and the neoplasm. Further than this,

[5] Shattock, S. G., Seligmann, C. G., and Dudgeon, L. S., *Proc. Royal Soc. of Med.*, 1910, iii, Path. Section, 127.

[6] Ehrlich, P., and Apolant, H., *Berl. klin. Wchnschr.*, 1905, iii, 871.

[7] Loeb, L., *Berl. klin. Wchnschr.*, 1906, xliii, 798.

[8] Russell, B. R. G., *Jour. Path. and Bact.*, 1910, xiv, 344.

[9] Apolant, H., *Ztschr. f. Krebsforsch.*, 1908, vi, 251.

Apolant[10] by implanting together tumor cells of various sorts has determined some of the factors in the adjustment of neoplasms to one another. But the possibilities have not been exhausted.

TECHNIQUE OF THE EXPERIMENTS.

Throughout the present experiments use has been made of tumor and embryo from the mouse. These have been chopped to a semi-solid consistence, and injected, alone or mixed, into the lateral subcutaneous tissue of other mice. The embryos were from 0.9 to 1.6 centimeters in length, that is to say, about eight to five days from term, at which period no difficulty is experienced in reducing them to a mass that can be readily injected through a needle. The tumors, except for the sarcoma already noticed, were adeno-carcinomata which had arisen and been propagated in New York mice. Care was taken to pick out "healthy" portions and these were reduced to the same pulpy state as the embryos. Intimate mixtures of the two in equal parts were made, and a drop or two of mouse serum added when the material could not be drawn into the syringe without it. This seldom happened. The amount used as a graft was 0.02 cubic centimeter, sometimes double this quantity. Observations were made upon twelve series, each consisting of ten to thirty mice.

CONDITIONS REGULATING THE MIXED GROWTH.

Growth of both elements in a mixed implantation does not always take place. A balancing of "avidity" is necessary, such as Apolant had recourse to in his mixtures of carcinoma and sarcoma, in order to prevent one from outgrowing the other. When embryonic fragments have been implanted with the Ehrlich sarcoma, the nodule that results after seven days consists almost wholly of tumor. By two methods, the cells of the embryo have been crowded out of competition. The tumor has proliferated so rapidly that its zone of active tissue has extended beyond the region of the embryonic

[10] In a work that has just come to my attention, Etiologia del cancro (Rome, 1909), G. Fichera describes briefly a similar experiment. The tumor he used (a sarcoma of the rat) did not influence the embryonic tissue to malignancy. At first the embryo and tumor grew together, but later the embryonic fragments retrogressed and the neoplasm predominated.

fragments, which, together with the neoplasm in their neighborhood, are soon overtaken by necrosis; and where it happens that both tumor cells and those of the embryo have survived side by side, the proliferation of the latter is in some way retarded, presumably through appropriation of their nourishment (plate X, Figs. 1 and 2).

When associated with a less rapidly growing neoplasm, or with one that possesses an abundant stroma, the fragments of the embryo develop more fully. Plate XI, Fig. 3, shows part of the growth from a mixture of adeno-carcinoma and embryo. The tumor, supported by a considerable stroma, grew almost as rapidly as the Ehrlich sarcoma, though with less tendency to necrosis; and the bits of embryo developed in its midst. Here the constitution of the nodule was such that the two tissues were separated by stroma and came into little immediate competition. Such competition as existed on the seventh day after implantation (when this nodule was examined) may be thought to have expressed itself in a demand on the host, not on individual capillaries. Earlier, before an adequate vascularization of the injected mass has taken place, a more direct rivalry for food presumably occurs. Certainly the fragments of embryo develop less perfectly in such mixtures than when implanted alone.

A delicate, slowly growing tumor introduced with fragments of embryo is rapidly outgrown and enveloped by them. Thus it happens that one finds islands of tumor deep in a nodule made up for the rest of embryonic tissue (plate XI, Fig. 4). These islands show no sign of degeneration to suggest that they are injured by a direct competition with the embryonic tissue about them. Nevertheless, the neoplasm fares badly in the end, because the retrogression and necrosis, which after a week or two overtake the mass of embryonic tissue, involve also the tumor within it.

Perhaps to this cause may be ascribed the failure of some attempts to utilize the angiotactic influences of embryonic tissue in the propagation of tumors. Attention has been called elsewhere[11] to the fact that embryo grafts evoke from the host a surprisingly

[11] Rous, Peyton, *Jour. Exper. Med.*, 1910, xii, 344.

rich vascularization. The idea suggested itself that this character might be turned to the support of delicate tumors unable on transplantation to elicit a stroma of their own. Accordingly, a mixture of embryonic tissue and a tumor of this nature was implanted in one flank of a number of mice, and tumor alone in the other. The latter grafts were the more successful.

RELATIVE SUCCESS OF TUMOR AND EMBRYONIC TISSUE.

Judging from the evidence of all the implantations, embryonic tissue of the mouse grows in a larger percentage of animals than the majority of mouse tumors, but in a smaller percentage than such active growths as the Ehrlich sarcoma. Even at the period of liveliest activity after implantation, it shows no proliferative energy comparable to the sarcoma's, or indeed to that of some carcinomata. It is true that these conclusions are based upon experiments made with embryonic tissue that was well along toward term; and it might be that cells taken at an earlier period would compare more favorably with neoplasm, both in transplantability and in proliferative energy. But M. von Tiesenhausen[12] has shown that the cells of very early embryos do not withstand transplantation even into the mother.

MORPHOLOGICAL RELATIONS IN THE MIXED GRAFT.

The morphological relations of growing tumor and embroyonic tissue in the mixed graft will now be taken up. Here cell differences prove, in the main, subsidiary to the mechanics of growth. The two tissues may be found in intimate association, and not infrequently one utilizes the other for its support (plate XII, Fig. 5). It is probable that in most of these instances the picture is somewhat misleading, since close examination discloses a scanty stroma separating the two. This stroma may be derived either from the host or from the embryonic tissues, but only in the latter case can the structural relation between tumor and embryo be considered very close. Where tumor islands are completely surrounded by proliferating embryonic tissue—which, as a rule, is

[12] von Tiesenhausen, M., *Virchows Arch. f. path. Anat.*, 1909, cxcv, 154.

penetrated very slightly by cells from the host[13]—there probably occurs some real adaptation of the connective-tissue cells of the embryo in support of the tumor. That this should take place is not strange, considering how tumor adapts normal adult tissue to its structural purposes.

DIRECT UNION BETWEEN THE EPITHELIUM OF TUMOR AND EMBRYO.

A yet more intimate relationship was observed in the *direct union of tumor epithelium with the stratified squamous epithelium of the embryo* (which latter on its growth in the new host tends to assume the adult form). A union of the sort was several times noted. The cells were not merely juxtaposed, but were joined to one another and somewhat intermingled at the meeting-point. In plate XII, Fig. 6, is shown a typical instance, and the accompanying outline drawings from serial sections indicate how the union came about (plate XIII, Figs. 7, 8, and 9). The bit of tumor figured is part of a larger mass, so the possibility that here one is dealing with a skin appendage can be ruled out. The drawings show that the tumor epithelium and that of the embryo proliferated along the rift between necrotic and healthy tissue. Thus, tongues of the two came end to end and a union followed. Such a result is not always obtained; the normal and the neoplastic cells may lie side by side yet quite discrete (plate XIV, Fig. 10). But when union does occur, the cells are sometimes intermingled to such an extent that the one tissue may be said to pass into the other, so far as this is possible of tissues quite unlike (plate XIV, Fig. 11). The carcinoma of Fig. 11 has long been propagated and has never shown any metaplasia to the squamous form.

Pictures of this sort may be looked for in vain through many sections which show an intimacy of the growing tumor and embryonic tissue. And this is easy to understand. Many mouse tumors form small closed acini, a circumstance which renders relatively slight the chance for their cells to meet directly the epithelial cells of the embryo. The very slight infiltrative tendency of mouse carcinomata in general, and the rapidity with which the epithelium of the embryo rounds into cysts after its implantation are other

[13] Rous, Peyton, *loc. cit.*

factors which tend to render uncommon a direct meeting of the two. Yet when such a contact does occur it may result in the union of quite dissimilar types of epithelium. An adeno-carcinoma may join and become directly continuous with a stratified, squamous epithelium possessing hair-follicles (plate XII, Fig. 6, plate XIII, Figs. 7, 8, and 9).

BEARING OF THIS OBSERVATION.

The bearing of this observation is not far to seek. For if such a union can occur between normal and cancerous tissues of different type, it would seem much more likely to take place between such as are similar, for example, between an epithelioma and the skin about it. A denuded surface between the two, caused by ulceration and kept clean, would give abundant opportunities for an extension and union of the sort here described, with a result in much more deceptive pictures. Furthermore, a carcinoma of infiltrative tendency might penetrate through the basement membrane to the normal epithelium and so come to unite with it. Indeed, several authors[14] have maintained on histological evidence that secondary union takes place frequently in both these ways; and a few have held to the extreme view that all instances of continuity between normal and carcinomatous tissue are secondary. Borrmann[15] has collected many histological specimens which more or less strikingly support this contention. Yet in the absence of direct evidence, the prevailing opinion among pathologists is still that the continuity between normal and carcinomatous epithelium denotes, as a rule, an origin of the latter from the former, a histological gradation between them rendering this in individual instances quite certain.[16]

The general truth of the concept may not be affected by the observations reported in this paper. While they prove that a secondary union between normal and carcinomatous epithelium does occur, yet they leave untouched the question of its frequency.

[14] Ribbert, Petersen, Liebert.

[15] Borrmann, R., *Ztschr. f. Krebsforsch.*, 1904, ii, 1.

[16] For a recent statement of the view, see H. H. Janeway, *Ztschr. f. Krebsforsch.*, 1910, viii, 403.

They show that with the union there may come some intermingling of cells, yet they do not demonstrate that a true histological gradation may also result from it. Nevertheless, it can hardly be disputed that where normal and carcinomatous tissues of similar type are continuous it might be most difficult to distinguish a mingling of the cells from a histological gradation between them. In view of this fact and of the occurrence of secondary union between normal and carcinomatous epithelium, as now demonstrated, the pathologist can no longer accept without circumspection the histological picture of continuity as proof of the origin of a carcinoma from a normal tissue.

SUMMARY.

Implanted mixtures of mouse embryo and tumor sometimes result in an excellent growth of both. To assure this, it is necessary that the tumor selected be one which proliferates slowly, because more active ones prevent the development of the fragments of embryo. Transplanted embryonic tissue (of the type used) does not, at its best, grow as rapidly or in so large a percentage of hosts as some tumors.

The morphological relations between tumor and embryonic tissue in the mixed graft are often intimate. Apparently either may adapt the other to its structural purposes. Occasionally a direct union takes place between cancerous epithelium and that of the embryo, with result in pictures suggesting an origin of one from the other. This observation has considerable significance in view of the current reliance upon just such histological data to prove that cancer arises from normal epithelium.

EXPLANATION OF PLATES.

PLATE X.

FIG. 1. Part of a mixed graft of mouse-embryo and mouse-tumor (Ehrlich sarcoma) after seven days in the host. The embryonic fragments have suffered in competition with the tumor and are in large part necrotic. One small cyst and a bit of cartilage of embryonic origin are present. That the host was in reality favorable to growth of the embryonic tissue is shown by Fig. 2.

FIG. 2. A graft of the same embryonic mass removed from the other side of the same host. There are present cysts lined by epithelium of various types and several well-grown pieces of cartilage.

FIG. 1.

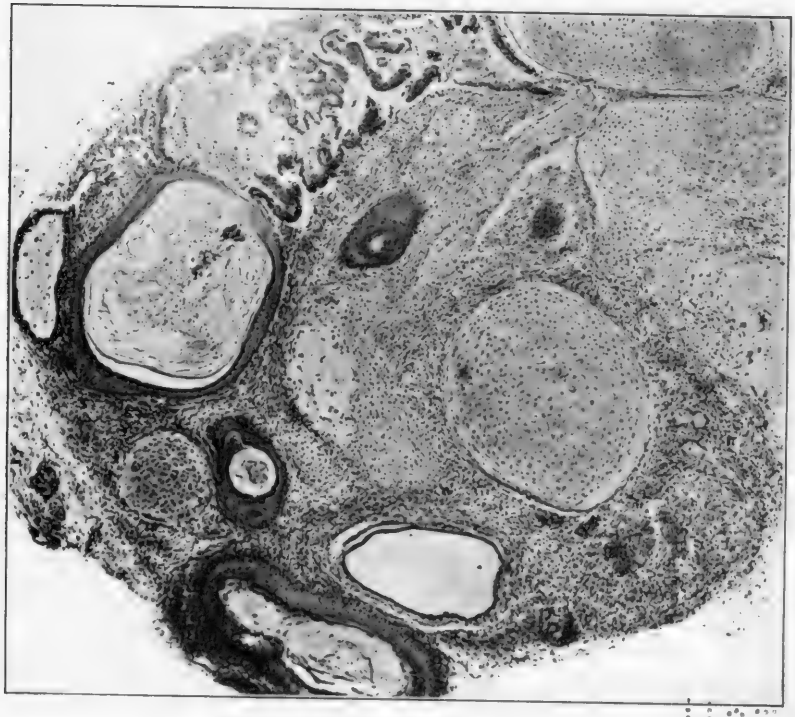

FIG. 2.

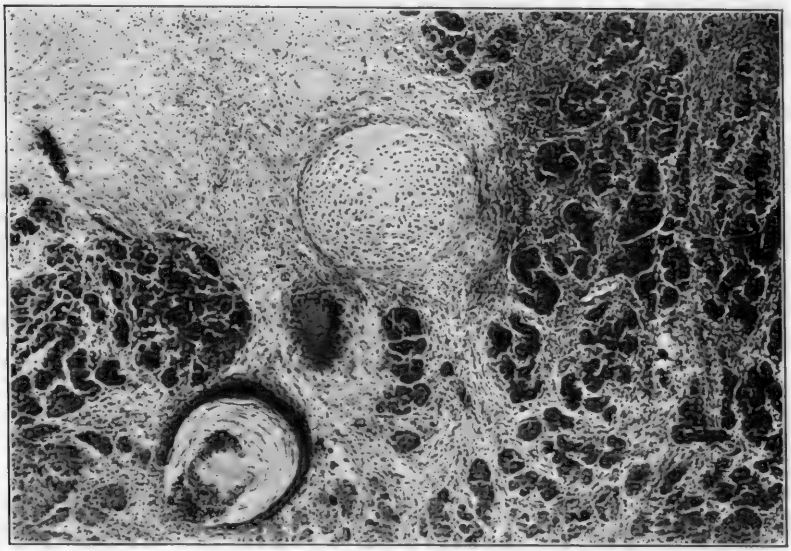

FIG. 3.

FIG. 4.

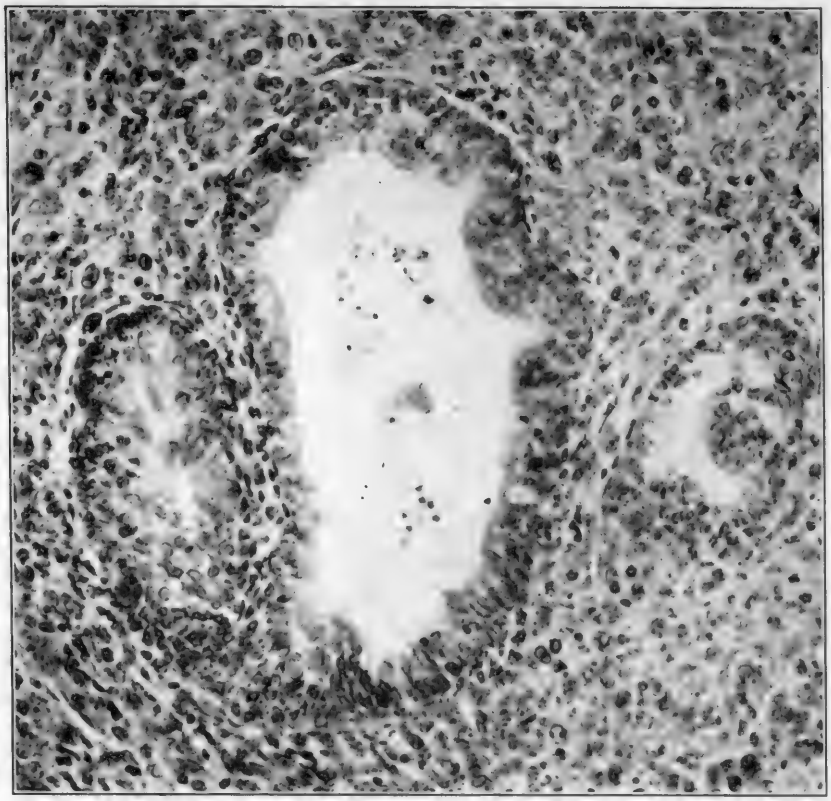

FIG. 5.

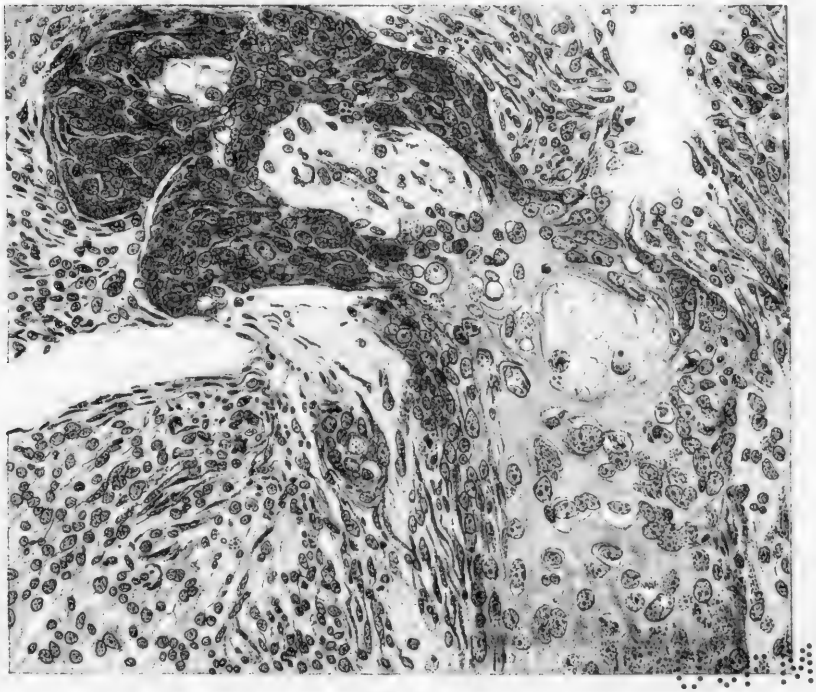

FIG. 6.

NOT

FIG. 7.

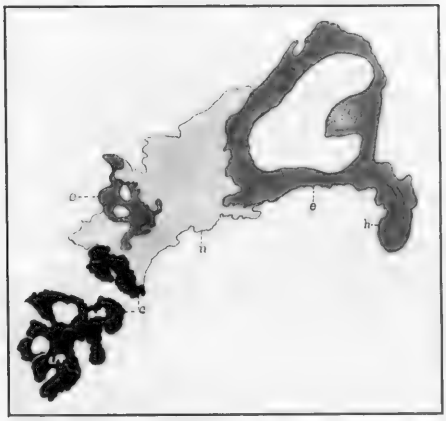

FIG. 8.

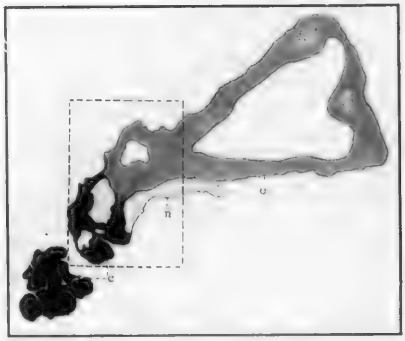

FIG. 9.

U of M

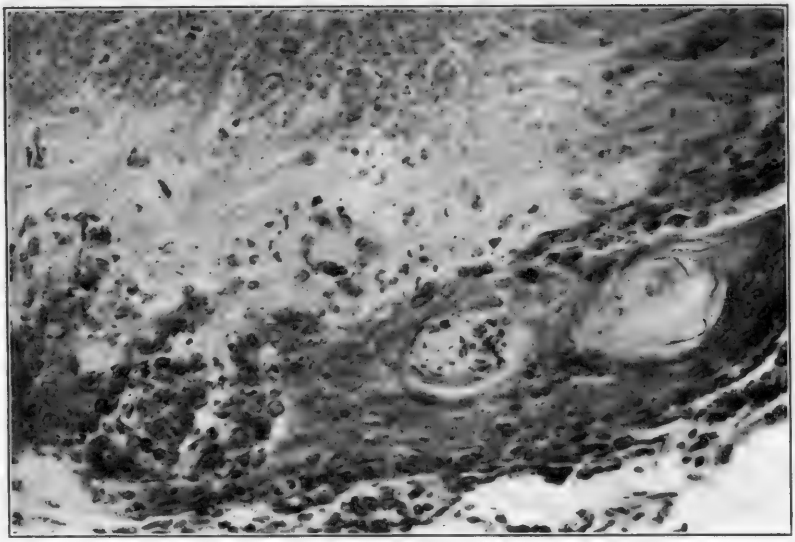

FIG. 10.

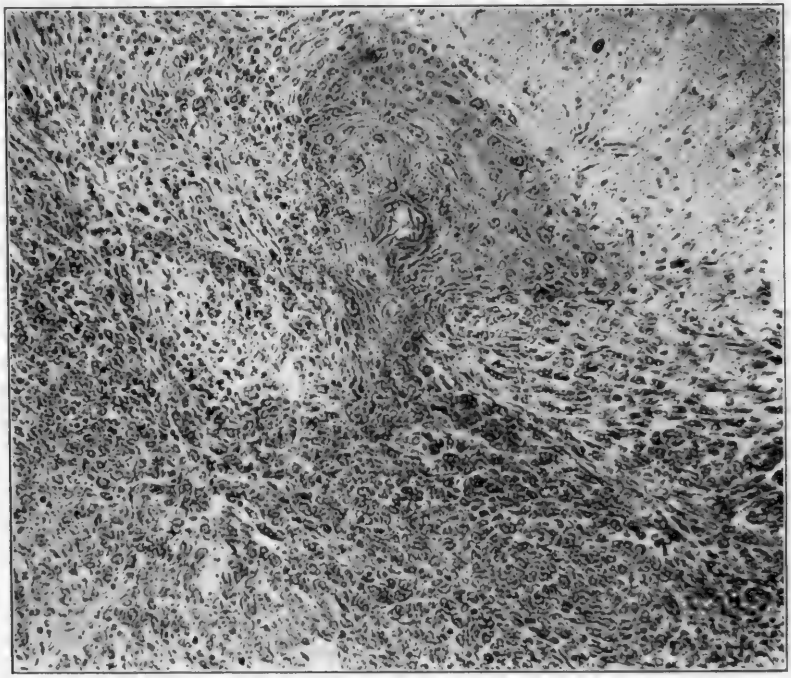

FIG. 11.

PLATE XI.

FIG. 3. Fragments of embryo growing in the midst of an adeno-carcinoma. Graft seven days in the host.

FIG. 4. Islands of tumor in the midst of a graft in which the embryonic tissues have predominated. Graft six days in the host.

PLATE XII.

FIG. 5. Cyst of embryonic origin utilizing sarcoma cells for its support.

FIG. 6. Direct union of squamous epithelium of embryonic origin and the epithelium of an adeno-carcinoma. The bit of tumor shown is part of a larger mass (plate ~~XII~~ Fig. 9). The possibility that here one is dealing with a skin appendage can be ruled out (see plate ~~XII~~, plate 13

PLATE XIII.

E. Squamous epithelium of embryonic origin, lining a cyst.
N. Necrotic tissue.
C. Adeno-carcinoma.
H. Hair follicle.

The three figures (Figs. 7, 8, and 9), drawn with a camera lucida from different points in serial sections, indicate how the union in plate XII, Fig. 6 came about. The tumor (black) and the squamous epithelium (gray) were separated by the necrotic mass (light gray). In proliferating, they extended along its margin in the rift between it and the healthy tissue and thus met end to end. In Fig. 8, a hair follicle is seen entering the wall of the cyst lined by squamous epithelium. In Fig 9, a dotted square encloses the area shown in plate XII, Fig. 6.

PLATE XIV.

FIG. 10. Squamous epithelium and carcinoma cells which have met but have not united.

FIG. 11. Union between an adeno-carcinoma and the stratified squamous epithelium of a mouse embryo. At the center of the photograph the carcinoma and squamous epithelium are united. Elsewhere connective tissue separates them. The carcinoma used has never shown any metaplasia to the squamous form.

ERRATUM.

In Volume XIII, Number 2, page 247, Fig. 6, lines 3 and 4, for "plate X" read "plate XIII."

THE EFFECT OF PREGNANCY ON IMPLANTED EMBRYONIC TISSUE.*

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PLATE XV.

The effect of pregnancy of the host on implanted tumor has been the subject of many observations; and it has been found that, in general, the growth of the neoplasm is retarded during gestation and may indeed cease.[1] The cause for this retardation is not known. It might be supposed, in view of the fact that sick or under-fed animals form unfavorable hosts for tumor, that the demands of the developing young upon the mother would constitute a sufficient explanation. But the assumption that gestation represents a nutritive tax upon the parent has been shown by Minot[2] to be unwarranted. In reality, "gestation does not represent a tax upon the parent but indeed a stimulus—it does not impede growth, but on the contrary favors it." So that this explanation cannot be accepted.

Comparing the behavior of transplanted embryonic tissue and transplanted tumor, I have been able to show that many of the characters deemed peculiar to the latter are shared by a normal tissue capable of growth on transplantation.[3] For example, an immunity to embryo similar to that for tumor can be brought about in mice, from which it appears that the two phenomena are merely instances of what may be called tissue immunity. The question now arises as to whether the observed influence of pregnancy of the

* Received for publication, November 4, 1910.

[1] Haaland, M., *München. med. Wchnschr.*, 1907, xliv, 713; Cuénot, L., and Mercier, L., *Compt. rend. Soc. de biol.*, 1909, lxvii, 736; Uhlenhuth and Weidanz, *Arb. a. d. k. Gsndhtsamte*, 1909, xxx, 434.

[2] Minot, C. S., *Jour. Physiol.*, 1891, xii, 97.

[3] Rous, Peyton, *Jour. Exper. Med.*, 1910, xii, 344.

host upon implanted tumor is not such another special instance of a more general phenomenon.

That the existence of pregnancy influences the growth of implanted embryonic tissue has been reported by several observers. The problem has indeed considerable interest in respect to normal physiology. L. Loeb[4] has demonstrated that a hormon controls decidual growth; and it is not beyond possibility that the development of the young *in utero* may be influenced in a similar way. Indeed, Askanazy[5] and Jentzler[6] state that pregnancy of the host seems to have a distinctly favoring influence upon the growth of experimental teratomas in white rats. Shattock, Seligmann and Dudgeon[7] studied grafts of fetal cartilage in rabbits that repeatedly bore young, without, however, observing that gestation exercised any special effect. Fichera[8] alone has attacked the problem systematically, and he records still another finding. When pregnant rats were hysterectomized and implanted with bits of their own embryos, the growth of the grafts was more marked, persisted longer, and the tissues developing were more diverse than in the non-pregnant, unoperated controls. When pregnant animals were inoculated with embryonic tissues from other individuals, growth of the grafts did not prove especially good, and in some instances in which the host carried a large litter, the grafts failed to grow and died.

METHOD OF AUTO-TRANSPLANTATION INTO PREGNANT HOSTS.

My own experiments were begun without knowledge of those of Fichera. They have already been reported in brief.[9] Mice and mouse-embryos were used as material. It was found possible to snare off from the gravid uterus at operation a portion containing

[4] Loeb, L., *Centralbl. f. allg. Path.*, 1907, xviii, 563; *Jour. Am. Med. Assn.*, 1908, i, 1897.

[5] Askanazy, M., *Verhandl. d. deutsch. path. Gesellsch.*, 1907, xi, 39.

[6] Jentzler, F., *Rev. méd. de la Suisse romande*, 1908, xxviii, 329.

[7] Shattock, S. G., Seligmann, C. G., and Dudgeon, L. S., *Proc. Royal Soc. of Med.*, 1910, iii, Path. Section, 127.

[8] Fichera, G., *Policlinico*, Sez. pratica, 1909, xvi, 692; Etiologia del cancro, Rome, 1909.

[9] Rous, Peyton, *Proc. Soc. Exper. Biol. and Med.*, 1910, vii, 71.

TABLE I.

Experiment	Animal	Embryos		Condition of Grafts						Microscopic		Remarks
				On Palpation				At Autopsy				
				First		Last						
		No.	Leng. in cm.	own	foreign	own	foreign	own	foreign	own	foreign	
I	A	7	1.0					×1½	×1½	Excellent	Good	
	B	6	1.3					×1	×1	Poor	Fair	Thin after operation. Wound healed slowly.
II	A	8	1.1					×1	×¾	Excellent	Fair	
	B	4	1.1					×1	×1	Good	Poor	Some hæmorge. into both grafts
III	A	10	1.0					×1½	×½	Excellent	Failed	
	B	3	1.6					×¾	×¾	Failed	Failed	
IV	A	6	1.2	2	2	3	2	×1	×1	Excellent	Fair	
	B	6	0.8	2	1	3	1	×1	×1	Good	Failed	
V	A	6	1.0					×1	×1	Fair	Fair	
	B	8	1.1					×1	×1	Fair	Fair	Thin and weak after oper't'n.
VI	A	7	1.6					×1	×2	Failed	Good	Own graft failed from hæmorrhage into it
	B	3	1.8	Large amount injected				×½		Failed	Good	Own graft shows vascularization but has not grown.

one or two embryos, without damage to the others, which continue to term. Implantation of the same material as that developing *in utero* was thus made possible, and it was unnecessary to resort to iso-transplantation. This constitutes a considerable advantage. For iso-transplantation of tissues gives, as is well known, very variable results. Possibly this circumstance alone accounts for the contradictory findings of previous workers. In our experiments, sixty-seven animals were operated upon, of which number fifty-three survived the term of experiment.

With ether as anesthetic, a median laparotomy was made, ligatures were quickly thrown about the uterus above and below the embryos wanted, and the included parts excised. Since the uterine vessels were within the ligatures there was practically no hemorrhage; and the animals recovered more quickly than from a Cæsarian section. The portion of the gravid uterus left behind was either not exposed in the wound or covered with sponges soaked in warm salt solution. The mice recovered well from operation, and abortion was infrequent. But to avoid complications a material was required for abdominal suture that could not be gnawed away. This was found in fine silver wire. Anything less resistant was removed by the animal with fatal consequences. When securely sutured a rapid union of the wound by first intention was the rule, and the new scar usually withstood the strain of parturition. Hemorrhage directly into the graft made it necessary to rule out some of the findings. Since sickness of the host has been shown to influence the fate of the grafts, results in animals that proved diseased (*e. g.*, sarco-sporidiosis, splenomegaly), or that suffered from post-operative complications (*e. g.*, abscess, hemorrhage) have been discarded.

The operation was performed five to nine days previous to term. The embryos are then 0.85 to 1.6 centimeters long, and still sufficiently soft to be easily chopped into a semi-solid mass that can be injected through a hollow needle. Of the well-mixed mass, 0.02 cubic centimeter was placed immediately after operation in the subcutaneous tissue of the host's side. Often two such grafts were placed at corresponding points. The material was injected from a syringe of small bore, and the dosage was well controlled. As

had already been shown,[10] a graft of the kind described, under favorable conditions, becomes vascularized and grows rapidly, reaching in the course of a week a bulk four to six times that injected, but retrogressing later with almost equal rapidity. In mice that proved unfavorable, the vascularization of the fragments fails to occur and they soon degenerate. The fate of the squamous epithelium, cartilage, and connective-tissue in a graft chiefly serves to indicate the "favorable" or "unfavorable" quality of a host. These tissues, so widely distributed in the embryo's body, are present in even very small portions of a chopped mass made therefrom.

EFFECT OF PREMATURE TERMINATION OF PREGNANCY.

The growth of the grafts, as noted on the seventh day, in operated mice from which all or part of the young had been removed, was often no greater than that observed in favorable alien hosts. In animals from which all the young had been taken, excellent growth was the rule; and it was found when the grafts were left undisturbed for two weeks that growth continued somewhat longer and tissue differentiation was somewhat greater than in non-pregnant aliens. Bony areas filled with normal looking red marrow were observed, and cysts lined with intestinal villi containing many goblet-cells, two features which rarely develop when the host is an alien.

The question arises as to whether these findings are the result of auto-transplantation, or due to some growth-substance peculiar to pregnancy. To decide the matter, pregnant mice were hysterectomized in pairs, and some of the embryo mass from each was implanted into both. In this way errors of deduction based on difference in viability of the material were ruled out.

Experiment I (*Table I*).—The grafts were injected into the subcutaneous tissue on each side of the host, and were palpated on the second or third day thereafter, and again on the sixth or seventh day, at which time the animals were killed. As a rule, these palpations were done without knowledge of the identity of the individual grafts, and silhouettes were made to express the size of the nodules felt. That these are essentially correct as an expression of relative

[10] Rous, *Jour. Exper. Med., loc. cit.*

sizes was shown by the findings at autopsy. The silhouettes made then are uniformly larger than those taken during life: first, because on palpation the grafts were felt as cylinders, whereas at autopsy they were found somewhat flattened; and secondly, because the resistance of a nodule has much to do with its apparent size on palpation. In the record of pair IV, the approximate transverse measurement of the little oblong nodules is given in millimeters. The autopsy silhouettes are throughout accompanied by a third dimension in millimeters. For example, XI means one millimeter in thickness. The silhouettes have been reduced to about three-fourths of their size, and in judging them the measurements of the third dimension should be similarly reduced.

The table shows that growth was, in general, more profuse in the mother than in an alien female in the same physiological state. There was also a second and better marked difference. In some of the aliens the grafts failed to obtain a supporting stroma and vascularization from the host tissues, or, in other words, they failed to "take." This I have elsewhere pointed out[11] to be of frequent occurrence in healthy, non-pregnant aliens. The records of some seventy non-pregnant aliens injected show a failure of the vascularizing reaction in about a third of the number. But grafts in the mother, as the present work demonstrates (see tables I and II), regularly "take" unless she be unhealthy or some accident has attended the injection. That sickness of the host suffices to prevent a "take" has been demonstrated for aliens, and was repeatedly found true for mother hosts as well.

The differences in the fate of grafts in the hysterectomized mother as compared with other similarly treated mice make it plain that the favorable state of the mother cannot be laid to the influence of the pregnancy just terminated. To attribute it to a hypothetical growth-substance associated with or increased during pregnancy, one would have to assume that this substance acts only on embryonic tissue from the host. But the known superiority of autotransplantation over iso-transplantation will account for the facts, and thus renders superfluous the assumption of a specific growth-substance to account for the phenomenon.

It may be asked whether the removal of a large part of the uterus incident to the operation in the experiment just described was not of importance in determining its results. The opportunity to test

[11] Rous, *Jour. Exper. Med., loc. cit.*

this point came to hand in connection with the observations upon mice only partly hysterectomized and still carrying young. Abortion occurred in several such cases soon after the operation; and in these animals deprived of young but retaining the uterus, the fate of the grafts in no wise differed from that in hosts completely hysterectomized.

EFFECT OF CONTINUATION OF PREGNANCY.

The influence on embryo grafts of the presence of developing embryos *in utero* was noted in a large series of animals. All of the transplantations in the series were auto-transplantations (table II).

Experiment II (*Table II*).—The table contrasts the findings in animals still gravid and in those from which the young had been completely removed. To facilitate comparison, the silhouettes obtained at autopsy are omitted. The embryos of different litters differed much in size, and this was taken into account in arranging the table, but, as the results show, there was no corresponding difference in viability of their transplanted tissue. Grafts of material from embryos 0.85 and 1.6 centimeters long behaved in the same way. At the microscopic examination, separate records were kept as to whether the graft was supported and vascularized by the host tissues, or, in other words, whether it "took," and whether it grew subsequently. In the case of Nos. 3, 4, and 6, the approximate transverse measurement of the little oblong nodules is given in millimeters. The silhouettes have been reduced to about four-fifths of their size, and the measurements expressing the third dimension should be similarly reduced.

It is made plain by the table that the presence of developing embryos *in utero* distinctly retards growth of the grafts. This is, in general, striking, though there are exceptions. Excellent growth occurred in one host that carried a single fetus, and good growth in another carrying four fetuses. But in most of the gravid animals the grafts after their insertion increased little or not at all in size. The findings agree in the main with those of Fichera, obtained by iso-transplantation. It is evident that between pregnant mice and mice deprived of young by hysterectomy there exists a considerable difference when they are used as hosts for implanted embryonic tissue.

The adverse influence of pregnancy of the host on embryo grafts is so striking that it is difficult to understand the conclusion of Askanazy and Jentzler, that pregnancy of the host constitutes a

TABLE II.

Complete Hysterectomy.

Partial Hysterectomy.

Remarks	Exp. No.	Embryos No.	Embryos Leng.	Microscopic Findings.	Days before measurement First	Days before measurement Last	Measurements First	Measurements Last	Measurements First	Measurements Last	Days before measurement First	Days before measurement Last	Microscopic Findings.	Embryos No. Out	Embryos No. Left	Embryos Leng.	Exp. No.	Remarks
	13	10	0.95	"Take" and excellent growth	3	6					2	6	"Take" - has grown slightly	2	3	0.85	26	
	15	6	0.95	"Take" - Fair growth.	3	6					2	7	"Take" - has not grown.	2	3	0.85	23	
	39	6	1.0	"Take" and excellent growth.	2	7					2	6	"Take" - has not grown. Ditto	2	5	1.0	37	Two grafts in the same animal.
	7	7	1.0	"Take" and excellent growth.	1	6					3	7	Both are "takes" and one has grown slightly.	1	2	1.0	52	Ditto.
Two grafts in the same animal.	49	7	1.0	"Take" - excellent growth. "Take" - fair growth.	2	7					2	6	Both are "takes" but have not grown.	2	3	1.0	36	Ditto One foetus macerated
Partial hysterectomy, aborted on 2nd day.	54	More than two.	1.0	"Take" - slight growth.	3	6			2	4	2	7	"Take" and excellent growth	2	1	1.05	3	
	11	8	1.05	"Take" - excellent growth.	3	6			+	1	2	6	No "take" - infection?	2	4	1.05	4	
	12	4	1.05	"Take" - good growth.	3	6					2	7	"Take" but slight growth	2	5	1.05	19	
Two grafts in the same animal.	31	6	1.1	"Take" - excellent growth. Ditto.	2	7					3	7	"Take" but very slight growth.	1	3	1.1	51	Two grafts in the same animal.

				good growth.									slight growth.				
Two grafts in the same animal.	31	6	1.1	"Take"- excellent growth. Ditto.	2	7			3	7	"Take" but very slight growth.	1	3	1.1	51	Two grafts in the same animal.
	6	6	1.15	"Take" and excellent growth	4	6	2 3		2	5	"Take" but no growth.	2	5	1.15	20	Ruptured abdom. wound in labor on fifth day.
Two grafts in the same animal.	44	4	1.3	"Take" but slight growth. Ditto	2	7			2	6	"Take" but slight growth. Fragments in fluid of small cyst.	1	4	1.2	35	Two grafts in the same animal.
	42	6	1.4	"Take" and excellent growth.	2	5			2	6	"Take" but has not grown. Ditto.	1	5	1.2	25	Ditto.
	17	5	1.6	"Take" and excellent growth	2	5			2	5	"Take"- has grown	1	4	1.3	34	
									2	5	"Take"- but has not grown.	1	4	1.4	38	Ditto.

favorable condition for experimentally produced rat teratomata. But they made very few observations, and they furthermore concerned themselves with grafts seated and vascularized before gestation began. It may well be that in special instances the pregnant state brings with it changes in local conditions favorable to the seated graft. Clinical records show that pregnancy may accelerate the growth of spontaneous tumors; and we have ourselves observed recently a striking example of the phenomenon in an inoculated mouse.

Systematic microscopic examination of grafts affected unfavorably by pregnancy brings out a new fact. It was found that they had not grown, but, on the other hand, had not died, as would have been the case in unfavorable aliens. When taken out five to seven days after injection and examined they were found vascularized and the individual elements were living, whereas grafts from unfavorable alien hosts, observed at this period, show an almost complete cell-death due to lack of vascularization.[12] The contrast with grafts from hysterectomized mothers is equally striking. The latter show seven days after implantation relatively large masses of cartilage, epithelial cysts distended with secretion and cast-off cells, and a connective-tissue approaching the adult type. Many signs of degeneration are present, in the cartilage especially (plate XV, Fig. 1). But the thin strands of grafted tissue removed from pregnant hosts are perfectly preserved, and are made up of minute bits of cartilage, nests of epithelium, and an immature connective tissue (plate XV, Fig. 2). These peculiarities of the embryo graft in the pregnant animal do not seem to have been observed before.

EFFECT AFTER PARTURITION.

There are many instances recorded in the literature showing that tumors which have long lain dormant may suddenly begin to grow. The question suggests itself whether embryo grafts may not be influenced to develop by the termination of pregnancy. To test this point, the course of grafts was followed in mice after parturition had occurred; and to relieve the mothers of extra physiological

[12] Rous, *Jour. Exper. Med., loc. cit.*

strain the young were taken from them. Under these circumstances grafts sometimes grew and persisted without degeneration at a time when in the hysterectomized mother retrogression has usually established itself. But the examples are not regarded as decisive and do not warrant a conclusion.

CONCLUSION.

1. Grafts of embryonic tissue obtained at operation and implanted in the mother, will grow well in case she no longer carries young. The growth is no more rapid than that in favorable non-pregnant aliens, but persists for a longer period without retrogression and results in a greater variety of tissues. The superiority of auto-transplantation over iso-transplantation is responsible for this fact. No evidence of a specific "growth-substance" peculiar to the pregnant state is furnished by the experiment.

2. When a mouse is implanted with embryonic tissue from her own uterus, and she still carries developing young, the fate of the grafts is very different from that just described. They are vascularized from the host but fail to grow or differentiate. Yet they do not die, and after pregnancy is concluded they may start to grow. The finding is strikingly like that noted by others of implanted tumor in pregnant hosts. It seems probable that some general factor affecting the growth of implanted tissues is here concerned.

EXPLANATION OF PLATE XV.

FIG. 1. Part of an embryo-graft removed from the hysterectomized mother seven days after implantation. To be compared with Fig. 2, which is of similar magnification.

FIG. 2. An embryo-graft removed from the partially hysterectomized mother six days after implantation. She carried during this period five developing young *in utero*.

The grafts in each case consisted of 0.02 cubic centimeter of embryo mass.

FIG. 1.

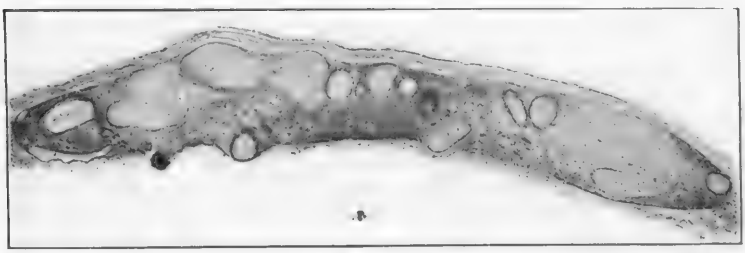

FIG. 2.

THE INFLUENCE OF MOUSE-RAT PARABIOSIS ON THE GROWTH IN RATS OF A TRANSPLANTABLE MOUSE SARCOMA.*

By R. A. LAMBERT.

(*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York.*)

PLATES XVI–XVIII.

In a former paper[1] it was shown that in a certain percentage of cases a successful parabiotic union between rats and mice was possible. The application of this procedure to tumor investigation appeared promising in view of Ehrlich's[2] interesting work in zigzag transplantation. His results may be briefly stated: a virulent mouse sarcoma when inoculated into rats grows rapidly for eight to ten days and is then absorbed. If reinoculated into mice at the height of growth the grafts are successful, and the cells continue to multiply with undiminished vigor. The resulting tumors may again be used for rat inoculations. Transplantations direct from rat to rat are, however, uniformly negative. Ehrlich explains this failure, as well as the limited growth in the first instance, through the exhaustion of a specific food substance, "X-Stoff," brought over from the mouse.

Accepting Ehrlich's hypothesis, it seemed conceivable that the deficient nutritive material might be supplied through a mouse-rat parabiosis, since it has been shown that in animals thus united substances in the body fluids of one animal pass fairly readily to the other.

Technique.—A free peritoneal anastomosis with wide skin and muscle apposition was made between young rats, weighing forty-five to fifty grams, and large "growing" or adult mice. Ad-

* This research was conducted under The George Crocker Special Research Fund. Received for publication, November 5, 1910.

[1] *Proc. Soc. Exper. Biol. and Med.*, 1910, vii, 94.

[2] Ehrlich, *Arb. a. d. k. Inst. zu Frankfurt*, 1906, No. 1, p. 77.

ditional sutures through the skin of the necks with bands of adhesive plaster about the bodies and tails formed sufficient fixation. More extensive bandaging interfered with feeding and promoted soiling of the wound by excreta.

Primary healing took place in a small number of cases (plate XVI, Fig. 1, and plate XVII, Fig. 2), a relatively wide granulating zone without skin union in a larger number, and only an approximation of the granulating surfaces in others. Histological studies are necessary to determine the extent and character of the healing process.

In preliminary experiments proof of an interchange of fluids was made by injecting certain substances into one animal and subsequently demonstrating these in the urine and body tissues of the other. Potassium iodide was shown to be present in the urine of the second animal even when there was an incomplete union of the apposed surfaces, the free peritoneal anastomosis being apparently sufficient to carry over the injected chemical. Trypan blue injected subcutaneously was noticeable in the peritoneal fluid of the opposite animal before general diffusion was apparent.

Parabiosis of mice and rats gave a high mortality in mice, the majority not surviving ten days. Hernia of the rat's intestines into the mouse occurred very frequently and was probably an important factor in causing death. The limited duration of parabiosis was a serious obstacle in the present investigation. To overcome this partially, the tumor inoculations were made on the day of parabiosis or several days before. Rats from experiments 78 to 84 were separated on the eighth day in order to insure a longer observation period, and three rats, from experiments 74 to 76 and experiments 64 to 70, were separated after the death of the mice. The tumors in two of the surviving rats were excised on the sixteenth and seventeenth days and studied histologically. Details of the microscopic findings are given in a later paragraph (see p. 261).

The tumor used in the present study was an Ehrlich mouse sarcoma, which in mice gives from 70 to 100 per cent. of takes. It pursues a rapid course and rarely undergoes retrogression. In normal young rats it gives a growth in about 40 per cent. of the animals, a definite nodule ten days after inoculation being regarded

as positive. As a rule, the tumors in rats at this time are larger than those of corresponding age in mice. After ten days the size may remain stationary for a day or two followed by a rapid retrogression with complete disappearance by the fifteenth or twentieth day. Two rat tumors were inoculated into mice for parallel studies but only one positive growth (out of twenty) was noted, and this was microscopic in size.

The following tables show the percentage of "takes" in five experiments representing thirty parabioses and controls.

TABLE I.

	Animals.	Survived.	Positive.	Negative.	Per cent. positive.
Experiments 57 to 63....	7	6	4	2	67
Controls	6	6	2	4	33
Experiments 64 to 70....	7	6	4	2	67
Controls	7	7	2	5	28
Experiments 74 to 76....	3	3	3	0	100
Controls	3	2	2	0	100

The inoculations in the above table were made on the day of the parabioses.

TABLE II.

	Animals.	Survived.	Positive.	Negative.	Per cent. positive.
Experiments 34 to 39....	6	5	4	1	80
Controls	5	5	2	3	40
Experiments 78 to 84....	7	6	4	2	67
Controls	7	6	2	4	33

In experiments 34 to 39 and 78 to 84, the inoculation preceded parabiosis by one and five days respectively.

TABLE III.

	Animals.	Survived.	Positive.	Negative.	Per cent. positive.
Parabioses	30	26	19	7	73
Controls	27	26	10	16	40

Table III combines tables I and II.

Chart 1 shows the increase and decrease in the size of the tumors in the experimental rats 78 to 84 and in their controls.

In this experiment fourteen rats, weighing forty to fifty grams, were inoculated with mouse sarcoma. Five and six days later

seven were united with mice, weighing twenty to twenty-five grams. The seven remaining animals were kept for controls. After eight days the rats and mice were separated, for it was desirable to observe them for a longer period and experience had shown that, after the eighth day, the mortality was high. In spite of the shortness of the parabiosis it was conceivable that sufficient interchange of fluid had taken place to influence the growth of the alien

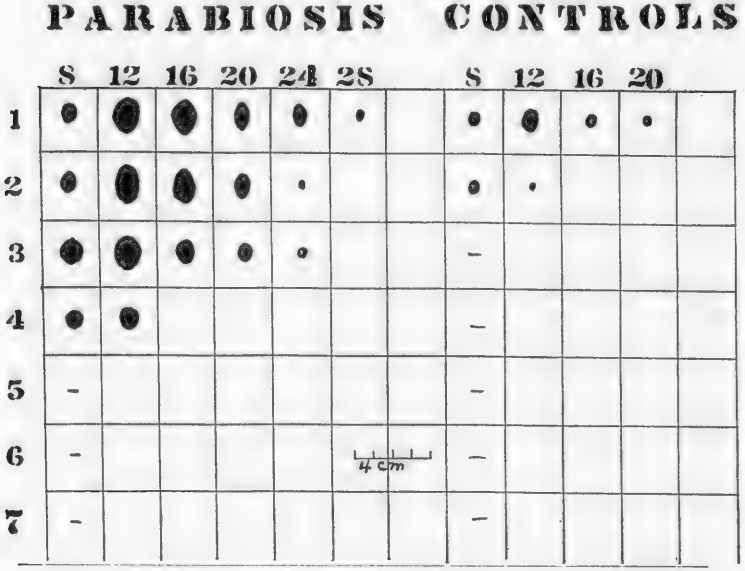

CHART 1. The figures 8, 12, 16, 20, 24, and 28, indicate the number of days after inoculation.

tumor. The tumors of three parabiotic rats were not absorbed until after the twenty-fifth day.

From table II it is seen that the percentage of takes in the parabiotic rats was 67 as contrasted with 33 in the controls. Since the inoculations preceded parabiosis by at least five days, it may be questioned whether this difference in percentage was not accidental. The larger size attained and the slow retrogression, however, can scarcely be explained other than through the favorable influence of

the union. Since the sarcoma does not give 100 per cent. of "takes" in mice of the size employed for parabiosis, some of the failures in parabiotic rats might be attributed to the insusceptibility of the associated mice. It did not seem desirable to test their susceptibility by simultaneous inoculation since another factor would thus have been introduced, one too, which according to our working hypothesis, might influence the results.

CONTROLS.

Before beginning the present series of experiments the growth of this tumor in normal rats was studied grossly and histologically. It was found that in the first ten days the appearances were practically the same as in the mouse tumors of the same age. After ten days, however, disintegration began in the central cells and rapidly involved the entire nodule. The blood vessels, however, remained patent for a time after the death of the cells. Absorption of the tumor was usually completed between the fifteenth and twentieth day.

A majority of the tumors in parabiotic rats was not studied histologically since it was desirable to determine the total duration of growth. Two tumors, however, excised on the sixteenth and seventeenth days, presented interesting pictures. Both tumors were removed from rats in which inoculation with tumor and the operation for parabiosis had been performed simultaneously and in both instances the rats and mice were separated on the eighth day. On the twelfth day the *first* tumor was 2.6 by .9 centimeters and remained stationary until excised on the sixteenth day. Sections showed that in the centre of the growth most of the tumor cells had disappeared, leaving the blood vessels—many with intact endothelium and filled with blood—standing out prominently. In the reticulum left by the disintegrated cells, normal and degenerating cells were scattered, and mitotic figures were frequently seen. Plate XVIII, Fig. 3, shows such a field.

In passing it may be suggested that this phenomenon can be explained by Ehrlich's hypothesis on the ground that the extensive necrosis of tumor cells, brought about by the limited supply of "X-Stoff," leaves the few surviving cells a proportionately larger share. This enables them to multiply actively until the same con-

dition occasions their death. In advancing this explanation it is assumed that through parabiosis there is circulating in the rat a certain amount of the specific nutritive substance.

The *second* tumor grew rapidly to the fourteenth day when it measured 1.6 by 1.2 centimeter. Before excision on the seventeenth day, the measurements were the same. Sections of this growth showed central necrosis, but less marked than in the first tumor. This softening evidently accounts for the lack of any increase in the size after the fourteenth day, since the presence of numerous dividing cells in the peripheral portions show that cell division had not ceased (plate XVIII, Fig. 4). Inoculations into mice were positive, confirming the microscopic evidence of vitality of the cells. The largest of the control tumors were excised simultaneously. Sections showed widespread granular necrosis, with no surviving cells.

CONCLUSION.

The results in these experiments seem to justify us in concluding that the growth of mouse tumors in rats is very definitely promoted by mouse-rat parabiosis, the percentage of "takes" being markedly increased, the rate of growth accelerated, and the duration of active growth extended to at least seventeen days.

These findings are in accord with Ehrlich's hypothesis, although, on account of the complexity of the factors involved in such experiments, other explanations may be advanced.

EXPLANATION OF PLATES.

PLATE XVI.

FIG. 1. Zone of union in a mouse-rat parabiosis of six days duration, showing perfect healing of all layers.

PLATE XVII.

FIG. 2. Mouse-rat parabiosis of eight days duration; x marks the point of skin union (approximate). The silk suture is visible.

PLATE XVIII.

FIG. 3. An area in the central portion of a sixteen day tumor in a rat of experiment 74 to 76, showing a patent blood vessel surrounded by normal and degenerating tumor cells, spaces left by dead cells, and three cells in stages of mitosis.

FIG. 4. Seventeen day tumor in a rat of experiments 57 to 63, showing mitotic figures.

FIG. 1.

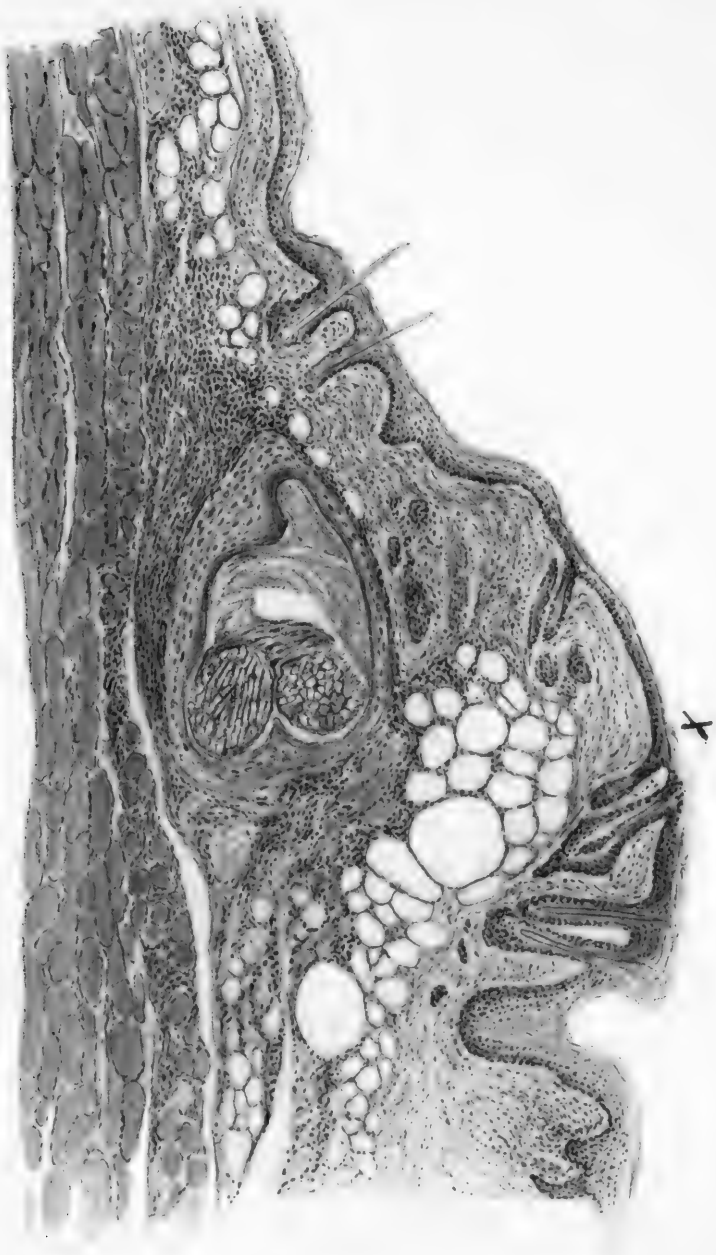

FIG. 2.

FIG. 3.

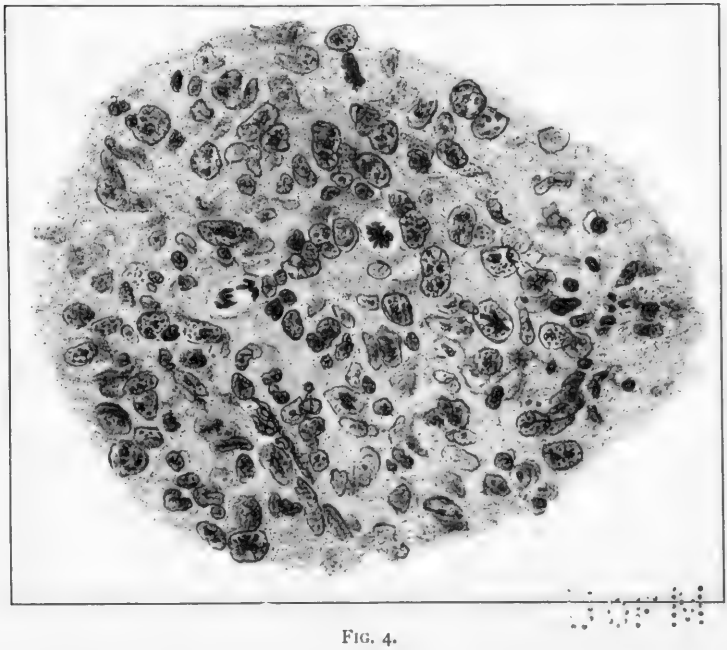

FIG. 4.

THE ÆSTIVO-AUTUMNAL PARASITE: ITS SEXUAL CYCLE IN THE CIRCULATING BLOOD OF MAN, WITH A DESCRIPTION OF THE MORPHOLOGICAL AND BIOLOGICAL CHARACTERISTICS OF THE PARASITE.*

By MARY ROWLEY-LAWSON, M.D.

PLATES XIX-XXIX.

The varieties of parasites commonly described as present in the circulating blood of cases of æstivo-autumnal malaria, are the small ring-forms, crescents and ovoids, including the male and female gametes. These latter forms are understood to be present but their sexual cycle is believed to occur *only outside the human body,* ordinarily in the body of the mosquito. I desire to report observations on all the forms mentioned above, beginning with the sexual cycle and a description of the development and morphology of the sexual forms as found in the circulating blood.

THE SEXUAL CYCLE OF THE ÆSTIVO-AUTUMNAL PARASITE AS FOUND IN THE CIRCULATING BLOOD OF ITS HUMAN HOST.

Study of many smears demonstrates that there can be distinguished not only in the mosquito, but *within the human body,* two sexual types of full-grown parasites (gametes). One, the male or microgametocyte, develops a flagellum (microgamete) which separates itself from the body of the parasite and enters the body of another type of parasite, the female or macrogamete, thus completing the process of fertilization.

Developmental stages of the different varieties of malarial parasites have been worked out in the mosquito by Ross, Bignami, Bastianelli, Grassi, and others. MacCallum while studying the blood

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of birds infected with the halteridium, and subsequently when examining the blood of æstivo-autumnal fever after its removal from the body, observed a flagellum break loose from a flagellated organism and penetrate the body of a non-flagellated parasite; this he interpreted as a process of fertilization.

My studies appear to demonstrate that not only is there differentiation into male and female elements, but that the process of fertilization and sporulation occurs in the circulating blood of human beings. Accordingly I shall trace, with constant reference to the microphotographs, the morphological stages in the sexual cycle of the crescentic æstivo-autumnal parasite in the circulating blood.

Staining Reaction of the Gametes (*Leishmann's Stain*).—The microgametocyte and the macrogamete, in their early ring-form stage, stain similarly; but as the parasite develops in size, the body of the microgametocyte stains more intensely blue than does the body of the macrogamete.

The nucleus of the microgametocyte stains a magenta-pink, while the nucleus of the macrogamete takes a very delicate pink color. The pigment is green. The capsules are usually achromatic but very occasionally one may be seen stained an almost imperceptible blue.

Encapsulation.—Many of the parasites, after the small ring-form stage, appear to be encapsulated. The capsules may be either opaque or semi-opaque to the red corpuscles, and when a precipitate has been thrown down with the stain used, the achromatic capsule shows clearly against a dark background. Plate XXI, Figs. 8 to 14, and plate XXIX, Figs. 25 and 26, show adult crescents with only a rim of the corpuscle (at "a") protruding from beneath the capsule of the parasite. In plate XXI, Figs, 1, 2, 6, and plate XXIII, Fig. 5, the corpuscle can be seen showing faintly through the capsules of the parasites. The *microgametocyte* appears to be protected by the capsule only during the period of its development, for no trace of it is seen after the parasite has reached the flagellating stage; but the *macrogamete* appears to be protected by this capsule during both its developmental stage and the period of sexual activity (after fertilization).

THE MICROGAMETOCYTE (MALE).

Development of the Microgametocyte.—The parasite from which the flagellum later issues develops from the familiar small ring-form parasite, gradually increasing in size, but retaining its spherical contour throughout until it is as large or larger than the red corpuscle. Throughout its developmental "round" stages it never reaches as large a size as does the macrogamete (female) (see plate XXIII, Figs. 1 to 7, giving stages in the developmental cycle of the microgametocyte.

Nuclear Substance of the Microgametocyte.—The chromatin material in the early developmental stages is more compactly arranged than that of the macrogamete. It increases rapidly in bulk as the parasite develops, and when the microgametocyte has reached the adult stage, the chromatin forms itself into a delicate filament and is seen in the form of a loose skein within the body of the parasite (plate XXIII, Figs. 7, 8, 9). In some parasites the nuclear material is arranged in the form of rods, connected by very slender threads, and closely resembles the chromosomes of a nucleus undergoing mitotic division (as is seen in plate XXIII, Fig. 8). As a rule, this latter arrangement of the nucleus occurs while the parasite is in the circular form.

The Flagellum of the Microgametocyte.—The flagellum emerges from the body of the microgametocyte, usually from the concave side and always after the parasite has assumed the crescentic phase of development. The extremity of the flagellum is generally clubbed (plate XXIII, Figs. 24, 25, 26). The curled and twisted appearance of the flagellum suggests active and varied motion and makes it difficult to state accurately its length; but some appear to be two to three times the length of the flagellating parasite. Occasionally a grain of pigment is seen in or on a flagellum after it has emerged from the parasite (plate XXIII, Fig. 16). In every case there appeared to be but one flagellum to each crescent. It is exceedingly rare to find a trace of the corpuscle in connection with a flagellating crescent. Plate XXIX gives a much clearer idea of the flagellated crescents than can be obtained from the uncolored photographs.

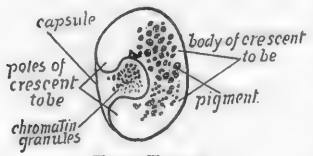
Pigment.—The pigment is green and in the form of dots and rods or both in the same parasite. It is less in quantity and arranged more compactly than is the pigment in the developing macrogametes. In the flagellating crescents, the pigment is generally centrally placed.

Cadaveric Parasites (after the Escape of the Flagella).—Some parasites show an entire absence of chromatin and contain more or less pigment (plate XXIII, Figs. 30 to 33). These are probably microgametocytes that have become cadaveric after the escape of the flagella. Most of these cadaveric parasites are short and thick, and nearly all of them show loss of staining affinity (degenerative changes).

THE MACROGAMETE (FEMALE).

Development of the Macrogamete.—The macrogamete (like the microgametocyte) develops from the small ring-form parasite, and gradually increasing in size without losing its circular contour, it reaches a larger size than does the microgametocyte before "opening out" into the crescentic form.

The Chromatin Substance and "Achromatic Zone" of the Macrogamete.—Soon after the appearance of the small ring-form stage of the parasite the nuclear material of the macrogamete breaks up into more or less finely subdivided granules and remains so throughout the development and sexual activity of the parasite. These granules are probably always contained in a circular or oval achromatic portion of the parasite. This achromatic portion, often difficult to show in the photographs, may be identical with the achromatic zone of the small ring-form parasite, and is generally situated in the part of the parasite between the poles of the crescentic body "curled up" in its capsule (text-figure 1). In many of the macrogametes the achromatic zone suggests a bladder-like sac containing the chromatin granules (plate XXV, Figs. 2, 3, 6 to 10, 16 to 18, 20 to 23, and plate XXIX, Figs. 16 to 21). This appearance would favor the theory of Grassi and Feletti that the colorless area in



TEXT-FIGURE 1.

the small ring-form parasite is a vesicular nucleus containing the chromatin granules of the parasite. If this achromatic zone of the macrogamete is a resistant secretion surrounding the nuclear material, would it account for the poor staining reaction of the nuclear granules? It might be well to state here that I was unable to see an achromatic zone for the protection of the chromatin granules of the microgametocyte, after the small ring-form stage.

Pigment.—The pigment of the macrogamete during its existence as a round body is more profuse and more scattered than is the pigment of the microgametocyte, but resembles it in color (green) and in being present in the form of dots and rods or both in the same parasite. It is generally situated in what will be the body proper of the macrogamete after it has "opened out" into the crescentic form and often tends to give the macrogametic round body the appearance of a curled-up crescent (plate XXIV, Figs. 19, 20, 21, 24, 25; plate XXV, Figs. 4, 6, 8 to 11; and plate XXVI, Figs. 1, 2, 3). Very rarely a few granules of pigment may be seen overlying the achromatic zone of the macrogamete (plate XXV, Fig. 6, plate XXIX, Fig. 18). When the macrogamete is preparing to sporulate, the pigment collects at the centre of the parasite (plate XXVI, Figs. 6 to 15).

Fertilization.—Before fertilization (i. e., when nothing resembling a flagellum can be made out in the macrogamete), the macrogametic round body generally shows a slight indentation in its outline (plate XXIV, Figs. 3 to 8) which probably corresponds to the clubbed ends of the crescent-to-be. Plate XXIV, Figs. 9, 10, 11, show an achromatic circular portion of the "round body" (at "*a*") which appears to be a part of the capsule; this portion of the parasite protrudes out from the rest of the body, slightly in Figs. 9 and 10, decidedly in Fig. 11. This circular portion of the macrogamete might be interpreted as a place of entrance for the reception of the flagellum.

The *flagellum,* or male fertilizing element, being in the form of an *unbroken filament* and staining a deep magenta-pink, can easily be distinguished from the nucleus of the macrogamete, or female, whose nucleus is always in the form of *granules* and stains a delicate pink. In plate XXV, Figs. 12 to 18, plate XXVI, Fig. 1,

and plate XXIX, Figs. 12 to 15, the flagellum can be seen partially within the macrogamete and partially outside of it. This appearance can be interpreted as meaning that flagella are *entering* the macrogametes. In plate XXIV, Figs. 19 to 25, plate XXV, Fig. 19, and plate XXIX, Fig. 16, the flagellum can be seen partially in that portion of the parasite situated between the poles-to-be, and partially in the body proper of the macrogamete; and in plate XXIX, Figs. 17, 18, the flagellum is seen in its entirety in the body-proper of the crescent-to-be, and fertilization is accomplished. After fertilization has taken place the nucleus of the macrogamete is still seen between what will be the poles of the crescent when it has opened out (plate XXIX, Figs. 17 and 18).

What is the ultimate fate of the flagellum after its entrance into the body of the macrogamete? As the macrogamete reaches its full maturity, after fertilization and before segmentation, the flagellum within it appears to lose its delicate, thread-like structure, becoming shorter and thicker and apparently less in quantity. This condition is shown in plate XXIX, Figs. 19 to 23. The inference might be, in the absence of evidence showing that it unites with the individual nuclear material of the macrogamete, that it is absorbed, for I do not remember having seen chromatin material beneath the pigment in any of the segmenting crescents. And the evidence seems to support the theory that the nuclei of the presegmenting parasites are derived from the macrogametes' own chromatin material. In the fertilized macrogametes but one flagellum was seen in each parasite.

MANNER OF REPRODUCTION.

Segmenting Crescents.—Several theories of the early observers concerning the segmentation of the crescents are more or less supported by my observations.

(*a*) *Budding.*—It is rare that a crescent is seen with protuberances at its periphery or on its surface suggesting gemmation. Such an appearance is shown in plate XXII, Figs. 25 and 26. At the present time this is not believed to be a process of reproduction.

(*b*) *Transverse Segmentation.*—This theory has been strongly

supported by Mannaberg. Plate XXII, Figs. 27, 28, 29, give a suggestion of transverse division. Fig. 27 shows two nuclei; but such form of division is rare. I believe that those photographed were the only ones seen in my specimens. No good evidence of transverse segmentation could be made out in the crescents from which the pigment had been removed (plate XIX, Figs. 34 to 37), and over a hundred were examined.

(*c*) *Spore Formation.*—For this form of segmentation in the crescent, I find more evidence than for the "budding" or "transverse division." Plate XXVI, Figs. 7 to 15, show segmenting crescents. In this form of segmentation, the act of reproduction ends the individual life of the sporulating crescent by using up all the protoplasm in the formation of spores or ring-bodies. The number of spores to each crescent averages from nine to twelve. The segmenting crescents were not seen attached to red corpuscles. Plate XXVI, Figs. 9 and 11, show such a clear outline to the segmenting crescents as to suggest the presence of a protecting membrane for the sporulating crescents. With one exception the chromatin granules were arranged at the concavity of the parasite. These granules stained a very delicate pink, as did the chromatin granules of the macrogametic round body.

It seems probable that the chromatin granules of the macrogametic round body "open out" (as seen at "*a*" in plate XXIX, Figs. 22 and 23) from their arrangement in the ball-like form (plate XXIX, Fig. 21), and then arrange themselves along the periphery at the concavity of the crescent (as seen at "*a*" in plate XXVI, Figs. 5 and 6). This process occurs probably just prior to the segmentation of the crescents, and the function of these chromatin granules may be to furnish the nuclei of the new spores. The arrangement of the chromatin granules of the fertilized macrogamete at "*a*" in plate XXV, Fig. 1, suggests the possibility of this being the ultimate fate of the chromatin granules of the macrogamete, for I can find no evidence that the chromatin granules of the macrogamete enter into the body proper of the parasite after it has reached maturity and opened out, preparatory to sporulation.

I believe that plate XXVI, Fig. 16, represents the spores from a segmenting crescent. The blue-staining protoplasmic ring portion

of the parasites appears more delicate than is usual in the spores of free segmenting tertian bodies, or in those of the æstivo-autumnal rosettes.

Pigment-bearing leucocytes were found in every smear containing segmenting crescents (see plate XXVII), and as melaniferous leucocytes are most numerous in the blood of other types of malaria at the time of segmentation of the parasites, their presence here would be contributory evidence of the true nature of the "segmenting" crescents.

The proportion of males to females during the period of sexual activity of the æstivo-autumnal parasite is remarkably equal, while in the tertian infections, the proportion of males to females is very small. This might be explained by the fact that the males of the æstivo-autumnal infections develop but one flagellum, while the males of the tertian infections give off several, provided, of course, that each female parasite receives but one flagellum, which up to the present time I have found to be the case in both æstivo-autumnal and tertian infections.

THE MORPHOLOGICAL CHARACTERISTICS OF THE ÆSTIVO-AUTUMNAL PARASITE AND ITS RELATION TO THE RED CORPUSCLE.

I. THE SMALL RING-FORM PARASITE.

The small ring-form parasites closely resemble the young parasites of the tertian and quartan malaria. They may be found free in the blood serum or attached to corpuscles. They are more often seen attached to the *periphery* of the red corpuscle than are the ring-forms of the other varieties of malaria. As a rule, they appear to contain no pigment; but careful examination of the ring-forms seen at the edges of smears where the parasites are often flattened out, show an occasional grain of pigment in their protoplasm.

Achromatic Zone.—These young forms are not really ring-shaped. The central portion is achromatic and only the outer zone takes up the stain, thus giving them the appearance of being ring-shaped. The youngest forms of the parasite which are found free in the blood serum immediately after segmentation (plate XIX, Figs. 1 to 8) show so little of the achromatic portion

that it is scarcely apparent and it is only after the attachment of the parasite to the corpuscle that this colorless portion becomes an appreciable part of the parasite. This central portion or achromatic zone may be so thin as to allow the corpuscle to show through or it may be opaque, as is seen in plate XX, Fig. 15, where it is shown stretched across the corpuscle from one parasite to another. This is also seen in plate XX, Fig. 14, where an appearance of budding is given to the corpuscle owing to the opacity of the achromatic portion of two parasites. Some of the early observers believed this achromatic portion to be a resistant secretion surrounded by an extremely delicate membrane (or cyst wall) for the protection of the nucleus; others believed it to be a vesicular nucleus containing the chromatin granules. There is no good evidence that the parasite is encapsulated during its earliest ring-form stage.

"*Loop Attachments.*"—Many of the small ring-form parasites show a more or less delicate, thread-like attachment (plate XIX, Figs. 15 to 25) in the form of a loop, arising from the body of the parasite, usually near its nucleus, and staining similarly to the body of the parasite. The function of this loop appears to be to assist the parasite in securing attachment to the surface of the corpuscle. In plate XIX, Fig. 15, each of the three parasites shows this delicate loop attachment. Occasionally a parasite having this attachment is seen free in the blood serum (plate XIX, Figs. 16 to 20). In plate XIX, Fig. 32, there is an "ovoid" or "contracted" crescent free from the corpuscle, with its pigment dissolved out, where the loop attachment can be demonstrated by careful search as a very delicate ring arising from the vicinity of the nucleus and extending just beyond the periphery of the parasite (dotted lines are put in with a pen just outside the loop to show its position).

In plate XIX, Figs. 22 and 26 to 31, small *round* rings are seen. These stained blue, but in them there was a complete absence of chromatin material. In this plate, Fig. 30 shows a thickening at one point corresponding to a nucleus. These rings might be interpreted as young parasites, the absence of chromatin being due to its forcible removal in spreading the smear, or resulting from an atypical segmentation such as is seen in the free segmenting tertian

parasites in plate XXVI, Fig. 21, where several of the ring-forms —*undoubtedly parasites*—(*oval* in shape) are seen to be without nuclei. More probably, however, the small round rings in the red cells are loop attachments broken loose from the ring-form parasites; for they never show an achromatic zone, and in plate XIX, Figs. 26 and 31, an exceedingly delicate thread can be seen connecting these rings ("loops") with ring-form parasites.

Chromatin, Its Irregular Distribution and Distortion in the Young Parasite.—The irregular distribution of the chromatin in the small ring-form parasite (shown in plate XX, Figs. 1 to 4, 6 to 11) is due, I believe, to technique while spreading the smear and illustrates how easily the chromatin may be subdivided. Most of the parasites showing the broken-up nuclei were found along the edges of smears made on slides and were generally seen in groups of several parasites whose nuclei were all *similarly distorted*. Most of the parasites were not close enough together for many to be included in good focus. Plate XX, Figs. 10 and 11, show nuclei distorted in circles, but the pictures lack contrast because the parasites had faded somewhat and made weak negatives, which had to be printed very dark to bring out the parasites. Here the distortion of the corpuscles shows violence in smearing. In plate XX, Figs. 1 and 2, the distorted nuclei show distinctly. Many microscopic fields were observed where groups of parasites showed a uniform, thread-like structure with nuclei at opposite poles. In plate XX, Fig. 8, two parasites are seen slightly drawn out of shape, the nucleus of one of these parasites at "*a*" is broken up into five granules of varying size, two of which extend beyond the protoplasmic periphery of the parasite. In plate XX, Figs. 12 and 13, parts of the nuclei of the parasites are drawn out in threads over the adjacent corpuscles.

The fact that the nucleus of the small ring-form parasite can be broken up or drawn out of shape (often beyond the body of the parasite) by technique, without distortion of the protoplasmic body of the parasite would seem to preclude the idea of the parasite being protected by a capsule at this period of its existence; it gives some evidence, however, that the parasite at this stage of development is attached to the external surface of the corpuscle, and not submerged beneath its surface.

Relation of the Small Ring-Form Parasite to the Corpuscle.—Evidence that the parasite is not intra-cellular but is attached to the surface of the corpuscle is shown in plate XX, Figs. 16 to 19, showing elongated parasites extending transversely across the red corpuscles.[1] They are undoubtedly young ring-form parasites drawn out when spreading the blood, *all in one direction without injury to or distortion of the red corpuscles.*

Multiple Infection by Small Ring-Forms.—Multiple infection of red corpuscles by young ring-form parasites is not uncommon in the blood of æstivo-autumnal malaria; but it is unusual to see more than three parasites in one corpuscle. Plate XIX, Figs. 9 to 15, show corpuscles infected with from three to six parasites. Some parts of the parasites do not show distinctly because it is impossible to get all the parasites in perfect focus at one time. Each nucleus represents a parasite.

Multiple infection of the corpuscles by older forms of the æstivo-autumnal parasites is exceedingly rare. I have never seen more than two mature parasites in one corpuscle in æstivo-autumnal malaria. It is quite possible that these young ring-forms destroy the corpuscle which they first attack, and then attach themselves to other corpuscles. The young ring-forms seen free in the blood serum with the loop attachments (plate XIX, Figs. 16 to 20), may result from such a condition. Certainly these parasites look older than those pictured in plate XIX, Figs. 1 to 8.

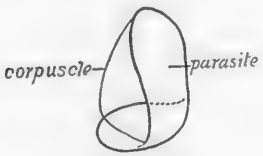
2. THE CRESCENT.

The shape of the crescent parasite is like that of a caterpillar whose extremities may both be blunt (plate XXI, Figs. 17, 20, 23), both pointed (plate XXI, Fig. 22) or one blunt and one pointed (plate XXI, Fig. 18). The crescent is wider than it is thick. This is shown in plate XXI, Figs. 22 and 23, where one end of the tape-like body is folded over itself (see also text-figure 2).

Relation of the Crescent to the Red Corpuscle.—The crescent is,

[1] *In the Bulletin of the Johns Hopkins Hospital*, 1904, xv, 22, I published an article called "Unusual forms of Malarial Parasites," wrongly interpreting these forms as stages in the life cycle of the æstivo-autumnal crescentic parasite, whereas they are obviously due to unequal pressure used while spreading the blood.

I believe, *extra-cellular* throughout the whole period of its development. By extra-cellular I mean attached to the external surface of the red corpuscle; but not incorporated within its substance or submerged beneath its surface. The parasite attaches itself to the corpuscle as a caterpillar wraps itself around a strawberry. This is especially well seen in plate XXI, Figs. 15, 19, 20, 21, 22, 23, 24, and 25. My interpretation of Fig. 18 is schematized in text-figure 3. The dotted oval represents the corpuscle, the outline of which, especially at "*a*," may be traced through the trans-



TEXT-FIGURE 2.

TEXT-FIGURE 3.

parent body of the overlying and embracing parasite. In plate XXI, Figs. 15 and 16, we can see the corpuscle projecting on either side of the crescent. Ordinarily, however, the corpuscle appears only on one side of the crescent (as the familiar "bib" or "apron," plate XXII, Fig. 7). The reason for this may be, that in the flow of the blood film under the cover-glass the crescent has the right of way, so to speak, because it is heavier and thicker than the corpuscle after its loss of substance. Therefore the crescent-parasite is first touched by the cover-glass or slide during the process of smearing, so that only slight pressure is necessary to start the crescent off, dragging after it the collapsed remains of the corpuscle. The presence of the "apron" on both sides of the crescent is rarely seen, and is much more difficult to explain.

Sometimes (as in plate XXI, Figs. 25, 26) the "apron" appears larger than the parasite. In Fig. 25, careful focusing showed that the parasite was partially detached from the corpuscle and doubled upon itself.

The So-Called "Double Contour" of Crescents.—Crescents have been described as having a double border which has been variously interpreted as a capsule, as a thickening of the protoplasmic border

of the parasite, as a remnant of the red blood corpuscle surrounding the parasite, and as one of the distinguishing characteristics of the quotidian æstivo-autumnal parasite. The smears from which the crescents with this apparent double-contour (plate XXII, Figs. 1, 2, 13, 14, 15, 17) were photographed were unevenly stained. In one part the eosin strongly predominated and it was in this eosin-area only that the crescents with the double contour, staining an intense eosin pink, were found. This border stained exactly the color of the red corpuscles in its vicinity, and in the portions of the smear where the double borders were found, the "aprons" or "bibs" were generally more deeply stained. It seems improbable that this double contour represents a capsule, for in every instance where I have observed capsules they were either achromatic or stained a most delicate blue, while the double border invariably stained an eosin pink. Achromatic capsules were seen in the same specimen and in the same eosin-zone, where the double contour crescents were found.

In the specimens studied, the double border appeared to be corpuscular remnants showing at the periphery of the parasite. In plate XXII, Fig. 18, note the appearance of the corpuscle at "a." The corpuscle showing at the ends of the parasite resembles the so-called double border.

Pigment in Crescents and Its Removal.—The pigment of the crescent is capable of being removed without apparent injury to the substance of the crescent. One finds pigment-free crescents along the edges of old smears where the xylol used for cleaning the oil from the cover-glass has soaked in between the cover-glass and slide and reached the smear. In a large blood-smear made upon a slide and covered only in part by a cover-glass, I have been able, by several washings with xylol, to remove the pigment from most of the crescents in the uncovered portions, while the crescents in the portion which was thoroughly protected by a cover glass from the action of the xylol, remained pigmented. It is highly probable that the xylol dissolved the pigment, for if it were removed mechanically it could not have been removed from any portion of the smear protected by the cover-glass. By reason of the fact that the nucleus of the "resting" crescent is usually beneath and obscured by the

pigment, we are unable to study its finer structure and position satisfactorily unless the pigment can be removed. Plate XIX, Figs. 32 to 37, show pigment-free crescents.

Study of pigment-free crescents show that, as a rule, the nuclear material is situated nearer to one end of the crescent than to the other, and is surrounded usually by an achromatic or white area, which area is often divided into two oval portions placed side by side like the glasses of spectacles (see plate XIX, Figs. 35, 36, 37). As a rule, there are several smaller achromatic or pale-stained areas on either side of those containing the nuclear material, along the long axis of the parasite. Comparison of many pigment-free crescents shows a suggestion of uniformity in the arrangement of these light areas.

3. ROUND BODIES AND OVOIDS.

The only *true round body is the developing crescent*, the other forms which are assumed by the adult crescent are pseudo-round bodies.

Pseudo-Round Bodies and Ovoids.—Many of the so-called round bodies and ovoids are:

(*a*) Crescents viewed from their convex side.

(*b*) Crescents bent on themselves.

(*c*) Contracted round bodies and ovoids (commonly found near edges of cover-glass smears).

These three classes will now be considered in greater detail.

Crescents viewed from their convex side are so seen when they are free from the corpuscle and curled up, either within a capsule or free from it. If seen in this position when attached to a corpuscle, the corpuscular substance can be seen only at the sides of the crescent (plate XXI, Fig. 15).

Crescents bent on themselves are seen in plate XXII, Figs. 30 to 34, and need no explanation other than that they are usually found at the edges of smears and their appearance may be due to technique in spreading the blood.

The contractability of the crescent may be seen by watching it on the warm stage. That it is *responsive to the stimulus of cold* is also known. Undoubtedly some of the crescents reach the edges

of the smear before dying and the contact with the cold glass may cause the parasites to contract, giving them the appearance of *ovoids* and *round bodies*. On the warm stage, adult crescents have been seen to assume forms similar to those shown in plate XXII, Figs. 1, 11, 12, 17, 21, 22. These round bodies are not so perfectly rounded as are the young, developmental, or *true round bodies* (plate XXII, Figs. 9 and 10, plate XXIII, Figs. 3, 4, 5). Notice the greater width of the contracted crescent as compared to that of the crescents *not* so contracted (plate XXII, Figs. 6 and 7). This result is what one would expect, for the crescents, in shortening their length, must necessarily increase in width.

The above mentioned contractile power of the crescent might explain some of the eccentric "aprons" seen in plate XXII, Figs. 6, 11, 12, 15, 16, and 17, for if a crescent were embracing a corpuscle (as is seen in plate XXI, Fig. 15) any contraction of the crescent, followed by a drawing along sideways in the smearing of the blood, would give a picture similar to that seen in the photographs.

Pigment in the Round Bodies.—In the developing or true round body, the pigment is seen usually nearer the periphery (plate XXII, Figs. 9 and 10) than the centre of the parasite. In the contracted crescents (plate XXII, Figs. 11 and 12), the pigment is seen nearer the centre of the parasite. In the crescents bent on themselves (plate XXII, Figs. 30 to 34) and viewed from the side, the pigment is seen nearer the periphery. A comparison in plate XXII, of Figs. 9 and 10, 11 and 12, and 30 to 34, will make the above clear. The arrangement of the pigment in the parasite is an aid to the correct interpretation of the type of round body.

4. ROSETTES AND SEGMENTING PARASITES FREE IN THE BLOOD SERUM.

In plate XXVI, Figs. 16 to 23, æstivo-autumnal rosettes, tertian pre-segmenting forms, and segmenting bodies free in the blood plasma are shown for the purpose of comparison. They were all photographed at the same magnification (× 2,000).

Æstivo-Autumnal Rosettes (Plate XXVI, Figs. 17 and 18).—As is well known, the æstivo-autumnal rosette is smaller than the tertian (plate XXVI, Figs. 19 and 20); it shows a more uniform

structure in the arrangement of the spores; and, as a rule, it contains fewer spores than does the tertian rosette, in my experience 12 to 18 as compared with 8 to 46 in the tertian rosette; and it does not enlarge the corpuscle as does the tertian parasite. In my specimens, the corpuscles containing æstivo-autumnal rosettes generally showed more loss of hemoglobin than did those containing tertian rosettes.

Pigment-free rosettes are rarely seen in the circulating blood. The pigment-free rosette shown in plate XXVI, Fig. 17, contains twelve spores. It was found in a specimen which also contained a rosette with pigment. The *Hemameba immaculata,* which is said to sporulate pigment-free, has been described as containing from six to ten spores (Grassi and Feletti, Marchiafava, Bignami, and others). In Fig. 19 (tertian segmenting body) no pigment is seen; but there is a circular area at "*a*" which may at one time have contained the pigment. In plate XXVI, Fig. 20 (tertian segmenting body), the pigment in the form of tiny rods appeared, under the microscope, to be attached to threads of blue protoplasm winding between the spores. This was more clearly shown in the stained specimen than would be possible to demonstrate in a photograph. In plate XXVI, Fig. 16, note the delicate protoplasmic æstivo-autumnal spores as compared with the heavier spores of the tertian parasites seen in plate XXVI, Figs. 21, 22, and 23. Plate XXVI, Figs. 16, 21 (atypical segmentation), 22, and 23, show parasites which apparently have segmented free in the blood plasma, as no trace of hemoglobin could be seen in connection with them. The threads seen connecting the small ring parasites in Fig. 16 were stained blue, similar to the blue of the parasite.

5. SCHUFFNER'S GRANULES AND MAURER'S DOTS.

Schüffner's granules are rather coarse bodies staining red and found in the protoplasm of red corpuscles infected with malarial parasites. Maurer's dots are in the form of tiny rings, staining red, also found in the protoplasm of red corpuscles infected by malarial parasites.

Maurer claims that the presence of Schuffner's granules in corpuscles is a valuable aid in the differential diagnosis of ter-

tian and æstivo-autumnal malaria in the early ring-form stage, and he states that these granules are present only in cells infected by the amœboid parasite, and that the tiny red rings (perniciosa macula) develop only in cells infected by the æstivo-autumnal parasite (see plate XXII, Fig. 24). In plate XXII, Fig. 23, Schuffner's granules are seen in a corpuscle infected by a crescentic parasite. In an early case of æstivo-autumnal malaria, where the blood contained only the small ring-form parasites, many red corpuscles containing parasites showed the coarse granules; others, the tiny red rings; and some, both forms of granulation. The fact that Schuffner's granules are seen in red corpuscles containing æstivo-autumnal parasites proves that this form of granulation is not confined to corpuscles infected by the amœboid parasite.

In plate XXII, Fig. 24A, the protoplasm of a red corpuscle infected by an amœboid tertian parasite (gamete) contains the dots (red rings) which Maurer described as being present only in corpuscles infected by the æstivo-autumnal parasite. While this form of granulation is more commonly found in corpuscles containing æstivo-autumnal parasites, it may be occasionally seen in corpuscles infected by the amœboid tertian parasite.

6. LEUCOCYTES.

In smears containing the active sexual forms, there was present an increase of eosinophiles. But, as a rule, in other smears I have found an increase of mononuclear cells, especially in smears containing crescents. Mitosis in the mononuclear cells of æstivo-autumnal malarial blood is not very rare. Plate XXVIII, Fig. 5, shows one in mitosis resembling the ordinary large lymphocyte. In the blood of tertian and æstivo-autumnal malaria, some unusual types of mononuclear cells were seen (plate XXVIII, Figs. 1 to 4), the protoplasm of these being white and filled with delicately staining blue granules of varying size. The nucleus is coarsely reticulated, showing considerable structure and staining a deep blue. Plate XXVIII, Fig. 2, shows one of these cells in mitosis.

7. PHAGOCYTOSIS.

This important function occurs in the majority of cases of æstivo-autumnal malaria and is performed in the blood both by the poly-

nuclear cells (microphages) and the mononuclear cells (macrophages). The most active phagocytosis in the blood is generally believed to be during the febrile paroxysm. I have found phagocytic cells most numerous at the time of sporulation and immediately after it. In certain smears (one containing almost exclusively the small ring-form parasites), the polynuclear cells predominated as phagocytes; but when the crescents began to appear the mononuclear cells were more numerous. The latter are usually found to predominate in the blood showing adult and segmenting parasites. The polynuclear leucocytes contained pigment and small ring-form parasites. The mononuclear phagocytes engulfed red corpuscles with and without parasites attached, also pigment, small ring-form parasites free from corpuscles, and, in one instance only, a crescent. Plate XXVII, Figs. 1 to 12, show phagocytic cells. These were found in the blood of æstivo-autumnal malaria and all but one (Fig. 1) contain inclusions. In cases of malignant æstivo-autumnal malarial fever, the brain capillaries are often loaded with parasites. Plate XXVIII, Fig. 7, shows a fixed endothelial cell of a brain capillary acting as a phagocyte and so loaded with blocks of pigment that it is impossible to make out any definite structure to the nucleus. Note that the capillary is filled with parasites attached to red corpuscles; the individual corpuscles with parasites attached can best be seen in plate XXVIII, Fig. 9.

8. ANEMIA.

In my experience, patients with æstivo-autumnal malaria generally show more anemia than do those infected by an equal number of the tertian parasites. In most of the specimens containing nucleated red cells, the blood showed all the characteristics of pernicious anemia. Plate XXVIII, Fig. 6, shows a gigantoblast in mitosis. The blood which contained active sexual forms showed a severe anemia, megaloblasts predominating over the normoblasts. In the blood of one case showing many old crescents, the corpuscles showed considerable loss of hemoglobin, nucleated red cells, enormous red corpuscles, and many off-colored and stippled cells were present.

GENERAL CONCLUSIONS.

1. The description of two distinct types of æstivo-autumnal crescentic parasites given by Craig in Osler's Modern Medicine (with difference in extremities, amount and form of pigment, double-outline, etc.), I have been unable to confirm.

2. The crescentic parasite is throughout its entire existence extracellular (i. e., not within the substance of the red corpuscle, but attached to its surface). It develops from the familiar small ring-form parasite, retaining a circular contour until fully matured.

3. The very young ring-form parasite has no capsule; the *macrogamete* has one during its development and sexual activity; while the *microgametocyte* has it only during its development.

4. The *Sexual Cycle*[2] of the crescent may occur in the circulating blood of its human host. This cycle is probably very rapid, for practically the whole cycle can be seen in a few smears taken from one puncture. The microgametocyte, or male, develops a flagellum (microgamete) which, *after the parasite has assumed the crescentic phase,* leaves the body of the parasite and enters the body of the macrogamete, or female, *during its existence as a round body.* Fertilization is then accomplished.

5. The microgametocyte, after the escape of the flagellum, shows evidence of degeneration and is probably cadaveric.

6. After fertilization, the macrogamete assumes the crescentic form, reaches the reproductive phase and undergoes sporulation, the product of which appears to be the small ring-form parasite. Renewed infection of the red cells then takes place.

7. That the sexual cycle is not seen oftener in the circulating blood of man may be due to one or more of the following reasons:

(*a*) The *process of reproduction* may be and probably is very rapid (some forms of protozoa are exceeded in rapidity of reproduction only by bacteria).

(*b*) The cycle may usually take place in some of the internal organs.

(*c*) It may be that the sexual cycle occurs in the peripheral blood only at intervals, when it becomes necessary for the parasite to renew its exhausted vitality.

[2] I have worked out the different stages of the developmental and sexual cycles of the tertian parasite and am now preparing the work for publication.

(*d*) The *occasional* occurrence of the sexual cycle in the circulating blood may be due to some slight chemical change in the composition of the host's blood serum.

8. I find no good evidence of conjugation-forms in æstivo-autumnal malaria. I found some of the young ring form parasites in close approximation or overlying each other. As the sexual cycle does take place in the blood of man, such a process of conjugation would seem unnecessary, unless it occurred as an atypical process.

The fact that the crescent is more resistant to quinine than the other varieties of malarial parasites might be explained by the presence of a capsule which appears to protect the parasite, certainly during the sexual phase and probably during the "resting" period (when not undergoing sexual changes). The youngest form of ring parasite shows no evidence of encapsulation. This may explain the fact that quinine is best administered just before the paroxysm when the parasite is undergoing segmentation.

I wish to acknowledge my great indebtedness to Drs. Theobold Smith and William T. Councilman, of Harvard University, for their advice and encouragement; to Dr. Stuart J. Lawson, of New London, Connecticut, I owe the photographs which have made it possible to demonstrate the results of my work; and to Dr. Richard C. Cabot, I am indebted for criticism and encouragement.

EXPLANATION OF PLATES.

PLATE XIX.

Magnification of Figs. 9, 10, 12, 13, 14, and 15, × 1,500; others, × 2,000.

FIGS. 1 to 8. Young ring-form parasites free in the blood serum.

FIGS. 9 to 15. Multiple infection of the red corpuscles by young parasites. Fig. 9 gives a good illustration of the achromatic zone. Fig. 15 shows three parasites fastened to the corpuscle by their loop attachments.

FIGS. 16 to 20. Parasites free in the blood serum, showing the loop attachment. In Fig. 19, the parasite has been pulled loose from the corpuscle and the loop has been broken.

FIGS. 21, 23, 24, and 25. Loop attachments of small ring-form parasites arising near the nucleus.

FIGS. 22 and 26 to 31. Small round rings, staining blue. Some are attached to the parasite by a very delicate thread, others are not. These are probably loop attachments broken loose from the parasites, or they may be chromatin-free ring-form parasites resulting from forcible removal of nuclei by technique in spreading the blood, or the absence of chromatin may result from an atypical

distribution of the chromatin during segmentation (see plate XXVI, Fig. 21, showing atypical segmentation of a tertian parasite).

FIG. 32. A pigment-free contracted crescent, showing a flagellum coming off, also the loop attachment arising near the nucleus in the form of a ring and extending down a little below the periphery of the parasite. Dotted lines just outside the loop attachment indicate its outline.

FIGS. 34 to 37. Pigment-free crescents. Two connected light areas can be seen at the center of the crescents in Figs. 35, 36, and 37. Notice the similarity in arrangement of the light areas of these crescents.

PLATE XX.

Magnification, × 2,000.

FIGS. 1 to 4 and 6 to 9 show small ring-form parasites whose nuclei have been broken up and distributed more or less over the bodies of the parasites. The breaking up of the nuclear material is probably due to technique, and, as the parasites can be injured without harm to the red corpuscles, the natural inference is that the parasites are attached to the surface of the red corpuscles.

FIG. 5 shows a peculiar achromatic portion in the body of the parasite, divided into two parts by a delicate line.

FIGS. 10 and 11. Nuclei of small ring-form parasites distorted to circular form by technique. They were found at the edge of a slide and the corpuscles show considerable distortion.

FIGS. 12 and 13. Threads of nuclear material drawn out from the nuclei of small ring-form parasites and extending beyond the bodies of the parasites over adjacent corpuscles. The parasites or corpuscles are not distorted. The fact that the nuclear material can be drawn out beyond the parasite would be evidence against the parasite's being protected by a capsule at this period of its existence, and would suggest also that it is attached to the surface of the corpuscle.

FIG. 14. An appearance of budding is given to the red corpuscles by the arrangement of the achromatic zones of two parasites, which zone is, in this instance, opaque.

FIG. 15. The achromatic zone drawn across a red corpuscle from one parasite to another. This zone must be composed of some non-staining substance and lie upon the surface of the corpuscle.

FIGS. 16 to 19. Young parasites drawn out, *all in one direction,* into elongated forms. Since this can happen without distortion of the corpuscle, the parasites *must be extra-cellular,* i. e., attached to the outside of the red corpuscles.

PLATE XXI.

Magnification, × 2,000.

FIGS. 1 to 7. Capsules enclosing the parasite. Note contrast of the achromatic portion when overlying red corpuscles.

FIGS. 8 to 14. Crescents still retaining their capsules and showing a portion of the red corpuscles emerging from beneath the achromatic portion of the parasite (see "*a*"). Fig. 10 (plate XXIX, Fig. 24) shows a gain of pigment in the capsule. The capsules here are opaque with the exception of Fig. 9, where part of the red blood corpuscle can be seen faintly through the capsule. Fig. 13 corresponds to plate XXIX, Fig. 25.

FIGS. 15 to 19. The *contour of the corpuscles can be traced through the crescent,* and the poles of the crescent are seen to extend beyond the outline of the crescent. This is evidence that the parasite is not within the corpuscle.

FIGS. 20 to 26. The crescents are wrapped round the corpuscles and seen from several view points. Here the *real concavity* of the crescent is seen and the remains of the corpuscle can be traced along it in Figs. 20, 21, 22, and 23. In Figs. 24 and 25, the crescent is seen from another point of view, and the corpuscle can be seen lying between the body of the crescent at "*a*" and its *clubbed* end. In Fig. 26, one end of the crescent has become loosened from the corpuscle and is bent back on itself.

FIG. 27. A crescent apparently embracing a corpuscle.

FIG. 28. A parasite on a red corpuscle which is surrounded by a dotted line stained blue like the body of the parasite. I do not know its significance.

FIG. 29. Shows pigment with some chromatin granules, overlying the edges of a crescent. There is also a pale blue-staining area, clearly belonging to the parasite but not an integral part of it, probably the capsule.

FIG. 30. A somewhat similar picture.

PLATE XXII.

Magnification, × 2,000.

FIGS. 1 and 2. Double contour crescents from which the pigment has been partially dissolved.

FIGS. 3 and 4 show the *real* as well as the *assumed* concavity of the crescent. See also plate XXI, Figs. 20 to 26.

FIG. 5. A crescent bent through its middle on its long axis, with nucleus at "*a.*"

FIGS. 6 and 7. Eccentricities of "apron" formation (remains of red corpuscles).

FIG. 8. Crescent with nucleus at one end and the pigment concentrated at the center.

FIGS. 9 and 10. Genuine round bodies or developing crescents, showing delicate clefts between which can be seen the achromatic substance, opaque to the corpuscle lying beneath. The pigment is nearer the periphery than the center of the parasites. In Fig. 9 it is in the *form of dots;* in Fig. 10 it is in the *form of rods.* Both parasites were found in the same smear.

FIGS. 11 and 12. Contraction crescents (crescents contracted into ovoids). The pigment is concentrated at the *center,* and in the form of rods and dots. Compare these parasites with the developing crescents (Figs. 9 and 10).

FIGS. 13 to 17. Eccentric arrangements of "aprons" (corpuscular remains).

FIGS. 18 and 19. Bulging of the corpuscle at the *convex* contour of the crescent, instead of at the concave.

FIG. 20. Crescent bent on itself with both poles on the same side of the corpuscle.

FIG. 21. Crescent attached to a red corpuscle and somewhat contracted, showing at "*a*" a portion of the cyst-wall, which is opaque to the red corpuscle beneath.

FIG. 22. A contracted round body, pigment centrally arranged, in the form of dots and rods. The parasite is shorter and slightly wider than those shown in Figs. 11 and 12.

FIG. 23. Schuffner's granules in a corpuscle *infected by a crescent.*

FIG. 24. Maurer's dots in the form of tiny red-stained rings in a red corpuscle which was infected by a small ring-form aestivo-autumnal parasite. The achromatic zone of the parasite and the loop attachment show well.

FIG. 24A. Maurer's dots (tiny red rings) in a red corpuscle infected by a tertian parasite (gametę).

FIG. 25. Crescent with protuberances at periphery resembling budding, described by some of the early investigators.

FIG. 26. Crescents with protuberances on the surface of the parasite, taking a deep red stain.

FIG. 27. Crescent with two nuclei; the pigment is arranged in a circle around each nucleus, giving the appearance of transverse segmentation.

FIGS. 28 and 29. Crescents showing contraction at the central portion of the parasites, giving them the appearance of preparing to segment transversely.

FIGS. 30 to 34. Crescents bent on themselves, suggesting round bodies. They were found on the edges of smears and their shape is probably due to technique.

PLATE XXIII.

Parasites of the crescentic sexual cycle. Microgametocytes (males) developmental stages. Flagellate bodies and cadaveric forms.

FIGS. 20, 21, and 22, magnification, × 1,500; other figures, × 2,000.

FIGS. 1 to 7. Stages in the development of the microgametocyte (male). Fig. 1 shows a light area surrounding the parasite—probably a partially transparent capsule. Fig. 5. The developing parasite shows an achromatic area at "*a*," opaque to the red corpuscle beneath, this area is probably part of the capsule. Fig. 7 (plate XXIX, Fig. 1) shows formation of the chromatin filament or flagellum before the parasite has opened out into the crescentic form.

FIG. 8. Nucleus of the parasite is in the form of rods (connected by almost invisible threads) resembling the chromosomes of a karyokinetic nucleus.

FIG. 9. The flagellum is in the body of the parasite. It can be seen lying parallel with the long axis of the crescent, and twisted into loops at the extremity of each pole of the parasite.

FIGS. 10 to 29. Flagellating crescents. Fig. 16 shows a grain of pigment on the flagellum outside the body of the parasite at "*a*." Figs. 17, 18, 19, are slightly contracted crescents. Figs. 24, 25, and 26 show the clubbed ends of the flagella. Fig. 29 shows a parasite with the pigment partially dissolved so that the emerging flagellum is clearer.

FIGS. 30 to 33. Cadaveric parasites with absence of chromatin; the bodies of 30, 31, and 32 are short and thick; Figs. 31, 32, and 33 show poor staining affinity (degenerative changes).

PLATE XXIV.

The sexual cycle. Macrogametes (females). Developing parasites and the phenomenon of fertilization. Magnification of Fig. 12, × 1,500; other figures, × 2,000.

FIGS. 1 and 2. Developing macrogametes, not fertilized (showing absence of flagella).

FIGS. 3 to 11. Older forms of developing macrogametes. Figs. 3 to 8 show a slight indentation in the outline of the parasite, probably corresponding to what will be the clubbed ends when the crescent has opened out. Figs. 9 to 11 show an achromatic, circular portion which appears to be a part of the capsule. This portion protrudes slightly in 9 and 10, and decidedly in 11. It may be the place of entrance of the flagellum.

FIG. 12 (× 1,500) to 18 and plate XXIX, Figs. 12, 13, 14, 15. Macrogametes, each containing its own nucleus in the form of chromatin granules at "*a*" and a flagellum, the entire length of which has not penetrated the macrogamete; a part of which can be seen outside the parasite at "*c*."

FIGS. 19 to 25 (plate XXIX, Fig. 16). The flagella have penetrated their length into the macrogametes (with the exception of Fig. 20, where a part of the flagellum is seen still outside the parasite at "*c*"), and the greater portion of the flagellum can be seen in that portion (at "*x*") of the macrogamete (the light area) which is situated between what will be the poles of the parasite after it has opened out into the crescentic form. Notice the large amount of pigment in these bodies and how in many of the parasites it accentuates the contour of a curled up crescent (Figs. 19, 20, 24, 25). In Fig. 21 (plate XXIX, Fig. 16), the macrogamete is seen attached to a red corpuscle.

PLATE XXV.

Fertilized macrogametes. Magnification, × 2,000. In most of them the flagellum has penetrated into the body proper of the macrogamete—that portion of the parasite which will be the body of the crescent when it has opened out.

FIG. 1 shows the fertilized macrogamete partially freed from pigment. At its periphery marked "*a*," the chromatin granules of its nucleus are grouped. Note that they extend the full length of the parasite. The grouping of the granules may be a preparatory stage for segmentation. The greater length of the flagellum is in the body proper of the parasite, but a slight portion which has not entered can be seen at "*c*."

FIGS. 2 to 5. Fertilized macrogametes, each with its own nucleus in the form of granules at "*a*" and the flagella at "*c*."

FIG. 6 (plate XXIX, Fig. 18). A fertilized macrogamete with some granules of pigment in the portion of the parasite generally occupied only by the chromatin granules. The pigment overlies the chromatin granules at "*a*" in the achromatic zone. The flagellum is at "*c*."

FIGS. 7 and 8 (plate XXIX, Fig. 17). Fertilized macrogametes in which, at "*a*," is seen the contour of a body containing the chromatin granules. Flagellum is at "*c*."

FIGS. 9 and 10 (plate XXIX, Fig. 20). Fertilized macrogametes showing the curled up crescent contour. Chromatin granules at "*a*," flagellum at "*c*."

FIGS. 12 to 22. Fertilized macrogametes with their chromatin granules at "*a*" and flagella at "*c*." Figs. 19 and 20 may be preparing to open out.

FIG. 23 (plate XXIX, Fig. 21). Fertilized macrogamete opening out, showing the contour of a round body containing the chromatin granules of the parasite at "*a*," situated at the concavity of the crescent. The flagellum has penetrated into the body of the parasite and shows loss of structure.

FIGS. 24 and 25 (plate XXIX, Figs. 22, 23). Fertilized macrogametes in which the body containing the chromatin granules appears to open out similarly to the method of opening out of the crescent. The flagella in the bodies of the macrogametes have lost their appearance of structure.

PLATE XXVI.

Fertilized macrogametes; pre-segmenting and segmenting crescents; rosettes; aestivo-autumnal and tertian parasites segmenting free in the blood serum. Magnification, Figs. 9, 12, and 13, × 1,500; the other figures, × 2,000.

FIGS. 1 to 4 (plate XXIX, Fig. 19). Fertilized macrogametes. Figs. 1 to 3 show a slight indication of structure in the body of the parasite. Fig. 2 shows the outline of the cystic wall at the opening between the two poles of the parasite. The line from "*a*" crosses it. The chromatin granules are at "*a*," the flagella at "*c*."

FIGS. 5 and 6. Chromatin granules are at the periphery of the parasite at "*a*." Pre-segmenting forms (?).

FIGS. 7 to 15. Segmenting crescents. Chromatin granules are usually situated at the concavity of the crescent. Figs. 9 and 11 show such straight unbroken outlines as to suggest the presence of a membrane around the crescents.

FIG. 16. An aestivo-autumnal parasite segmenting free in the blood serum. The appearance of the spores suggest that they arise from a crescent.

FIG. 17. Pigment-free rosette of aestivo-autumnal malaria (12 spores) found in a smear containing a rosette with pigment.

FIG. 18. Ordinary aestivo-autumnal rosette, the nuclei somewhat distorted in smearing the blood (18 spores).

FIG. 19 and 20. Tertian segmenting bodies (20 and 24 spores respectively) shown to compare size, arrangement, and number of spores with those of the aestivo-autumnal parasites. Fig. 19 shows absence of pigment, but there is a very faint area appearing at "*a*" which may have contained pigment at one time. Fig. 20 shows that the corpuscle is infected by a young ring-form parasite at "*a*" as well as by the segmenting parasites.

FIGS. 21 to 23. Tertian parasites segmenting free in the blood serum. For want of space, not all of the spores in Figs. 22 and 23 are shown. Compare these spores with those of the æstivo-autumnal segmenting body. Notice the *atypical distribution of chromatin* in Fig. 21, many of the rings being without nuclei.

PLATE XXVII.

Phagocytosis by the leucocytes in æstivo-autumnal malaria; magnification, × 2,000.

FIG. 1. Phagocytic lymphocyte or macrophage with its capturing processes out.

FIG. 2. Small lymphocyte containing pigment.

FIG. 3. Degenerated crescentic parasite in the broken-off process of a macrophage.

FIGS. 4 and 5. Large mononuclear cells (macrophages) containing red corpuscles and pigment clumps.

FIG. 6. Lymphocyte containing red corpuscle.

FIG. 7. Lymphocyte, with kidney-shaped nucleus, containing two small ring-form parasites at "*a*."

FIGS. 8 and 9. Mononuclear cells (macrophages) containing pigment.

FIG. 10. Polynuclear leucocyte or microphage, containing two blocks of pigment at "*a*."

FIGS. 11 and 12. Polynuclear leucocytes (microphages) containing small ring-form parasites at "*a*."

FIG. 12. The polynuclear cell appears to be putting out a process at "*c*" to capture the free parasite at "*d*."

PLATE XXVIII.

Magnification, × 2,000.

Peculiar mononuclear cells, whose protoplasm is white, containing coarse granules of varying size, and staining a delicate blue. The nucleus shows much structure and is coarsely reticulated, staining a dark blue. Fig. 2 shows this cell in mitosis. I have seen this type of cell in several smears taken from severe cases of æstivo-autumnal and tertian malaria.

FIG. 5. Lymphocyte in mitosis. Many such cells undergoing mitosis were found in the smears containing active sexual parasites.

FIG. 6. Gigantoblast in mitosis. Found in a smear where the blood showed all the characteristics of pernicious anemia.

FIG. 7. A brain capillary, filled with corpuscles with parasites attached, whose fixed endothelial cell at "*a*" is seen acting as a phagocyte. The protoplasm of this cell is so loaded with pigment blocks as to hide completely the structure of the cell nucleus.

FIG. 8. A group of young æstivo-autumnal parasites found free in a smear made from the brain.

FIG. 9. A brain capillary in which can be seen the outlines of the red corpuscles with parasites attached to them.

PLATE XXIX.

Magnification of Figs. 5, 6, and 12, × 1,500; the other figures, × 2,000.

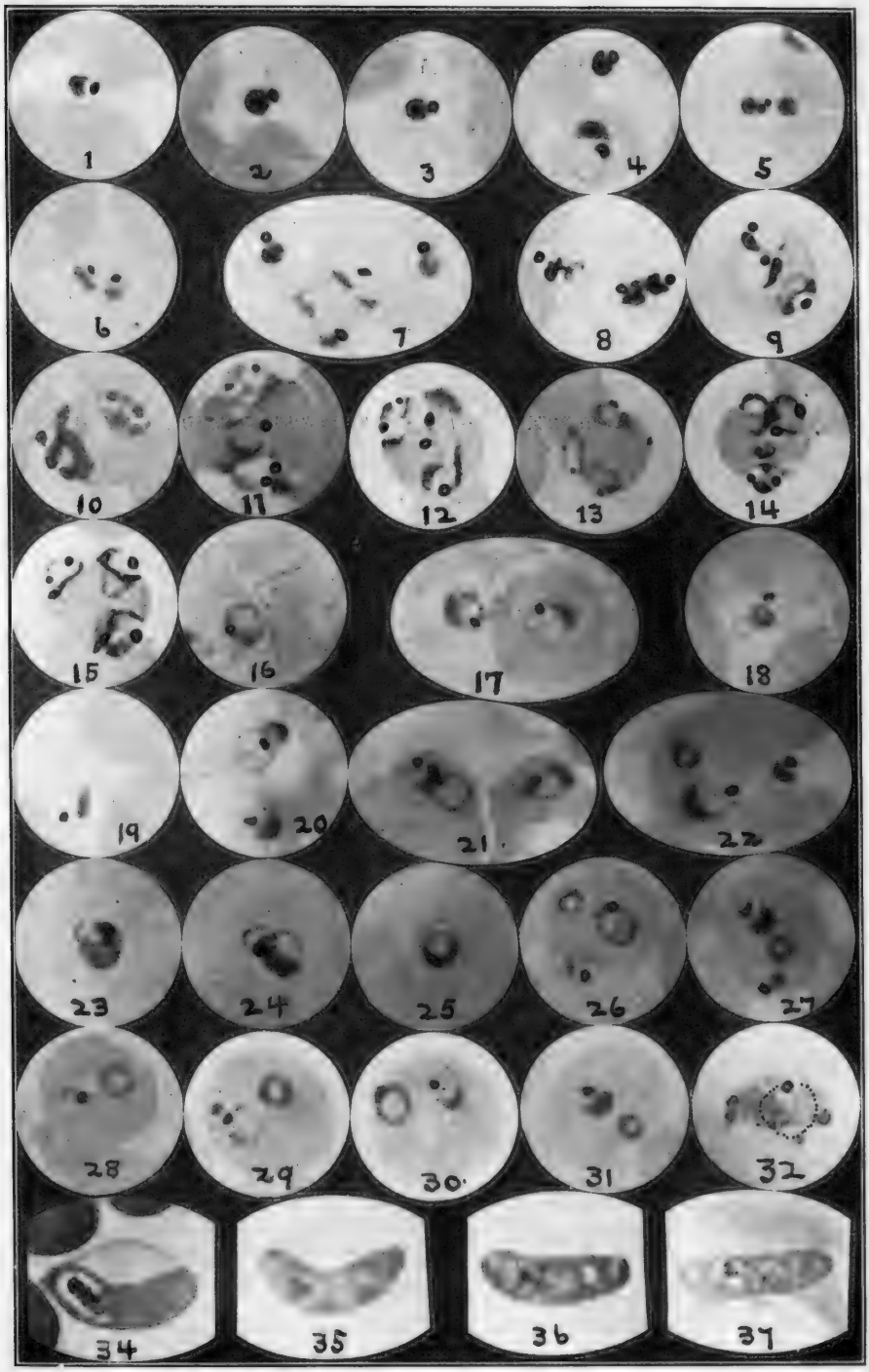
These pictures are photographs printed just enough to show structure and then colored in order to obtain greater accuracy than would be possible with free-hand work. The macrogametes should be colored a more delicate blue, and their chromatin granules a paler pink. Facsimiles of all the colored pictures are shown in the uncolored photographs for the purpose of comparison.

FIG. 1 corresponds to plate XXIII, Fig. 7. A microgametocyte (male) not yet opened out from its circular developing form, but with its chromatin already formed into a delicate filament, lying in a loose skein in the parasite, preparatory to flagellation.

FIGS. 2 to 10 correspond to plate XXIII, Figs. 17, 18, 19, 20, 21, 13, 14, 25, and 26. Flagellating crescents. Figs. 9 and 10 show the clubbed extremities of the flagella.

FIG. 11 corresponds to plate XXIII, Fig. 30. A cadaveric crescent after escape of the flagellum.

FIGS. 12 to 15 correspond to plate XXIV, Figs. 12, 14, 16, 17. Macrogametes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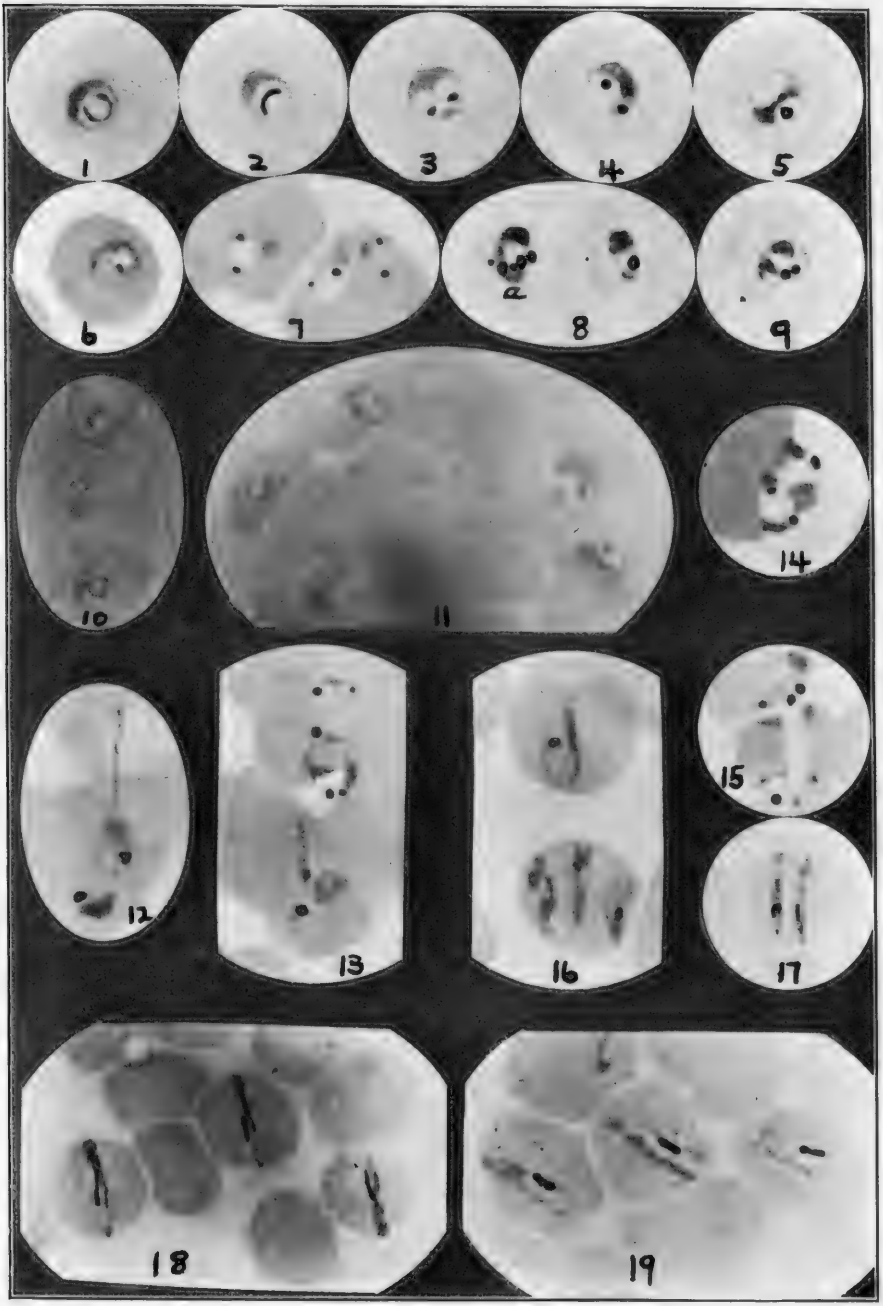
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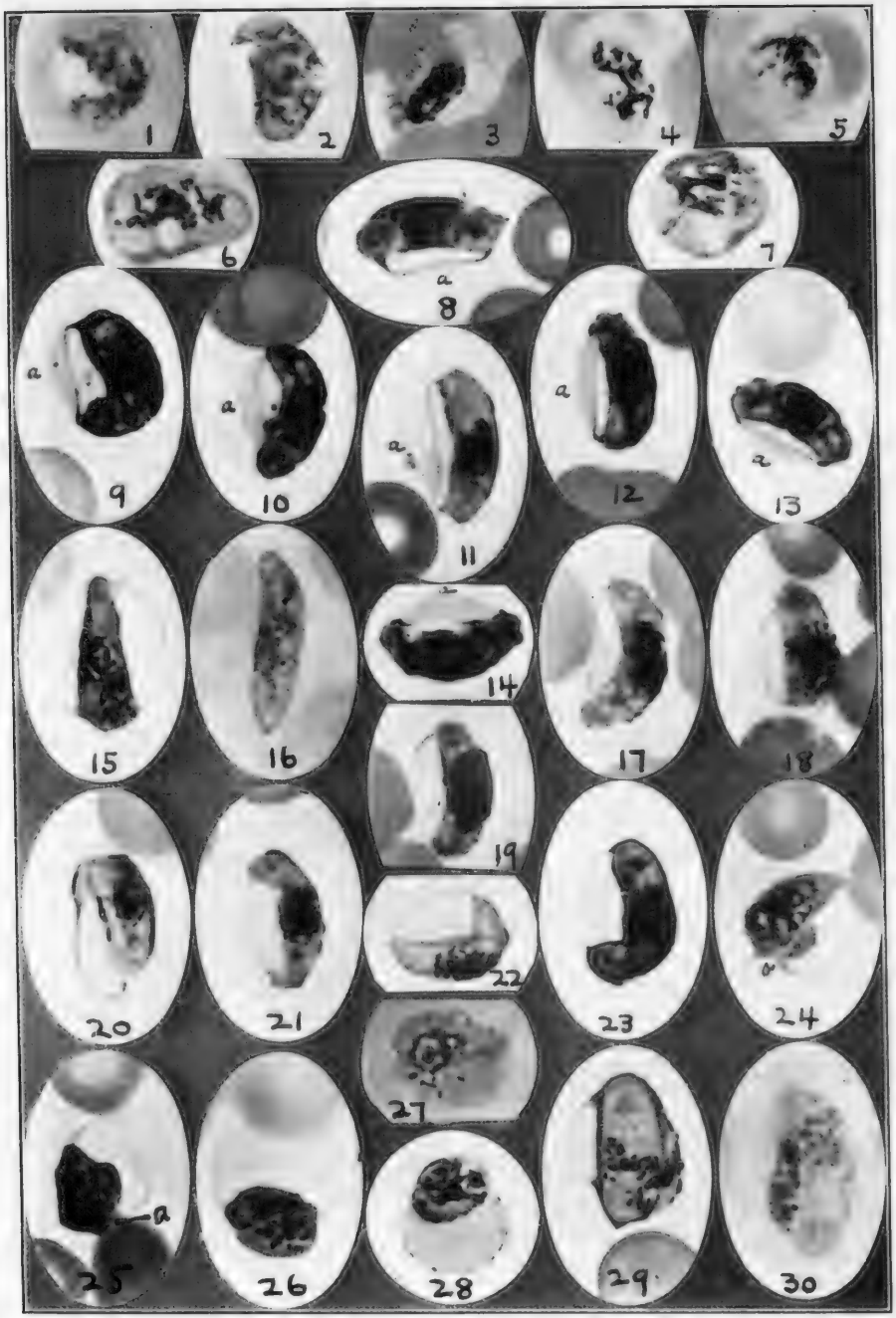
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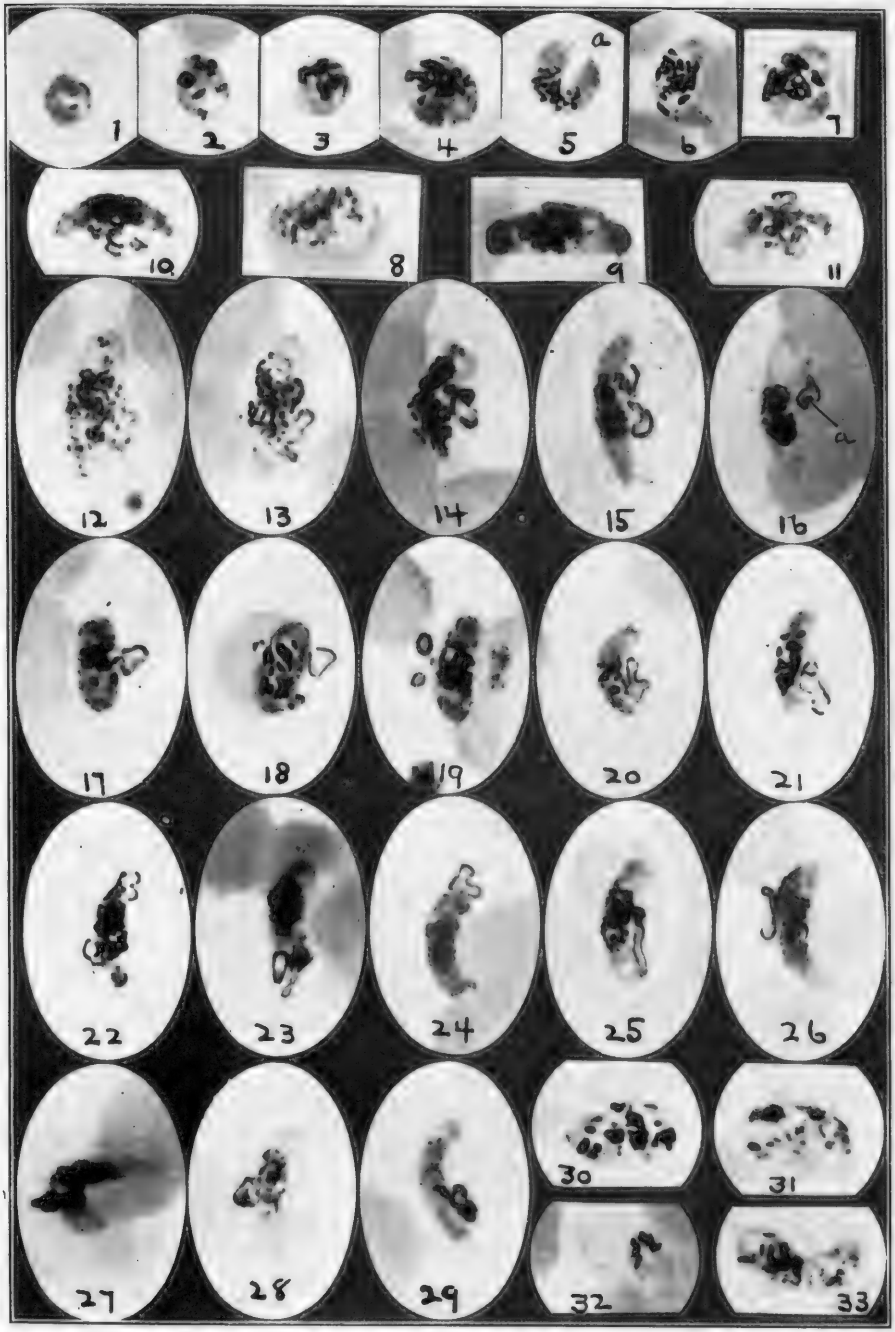
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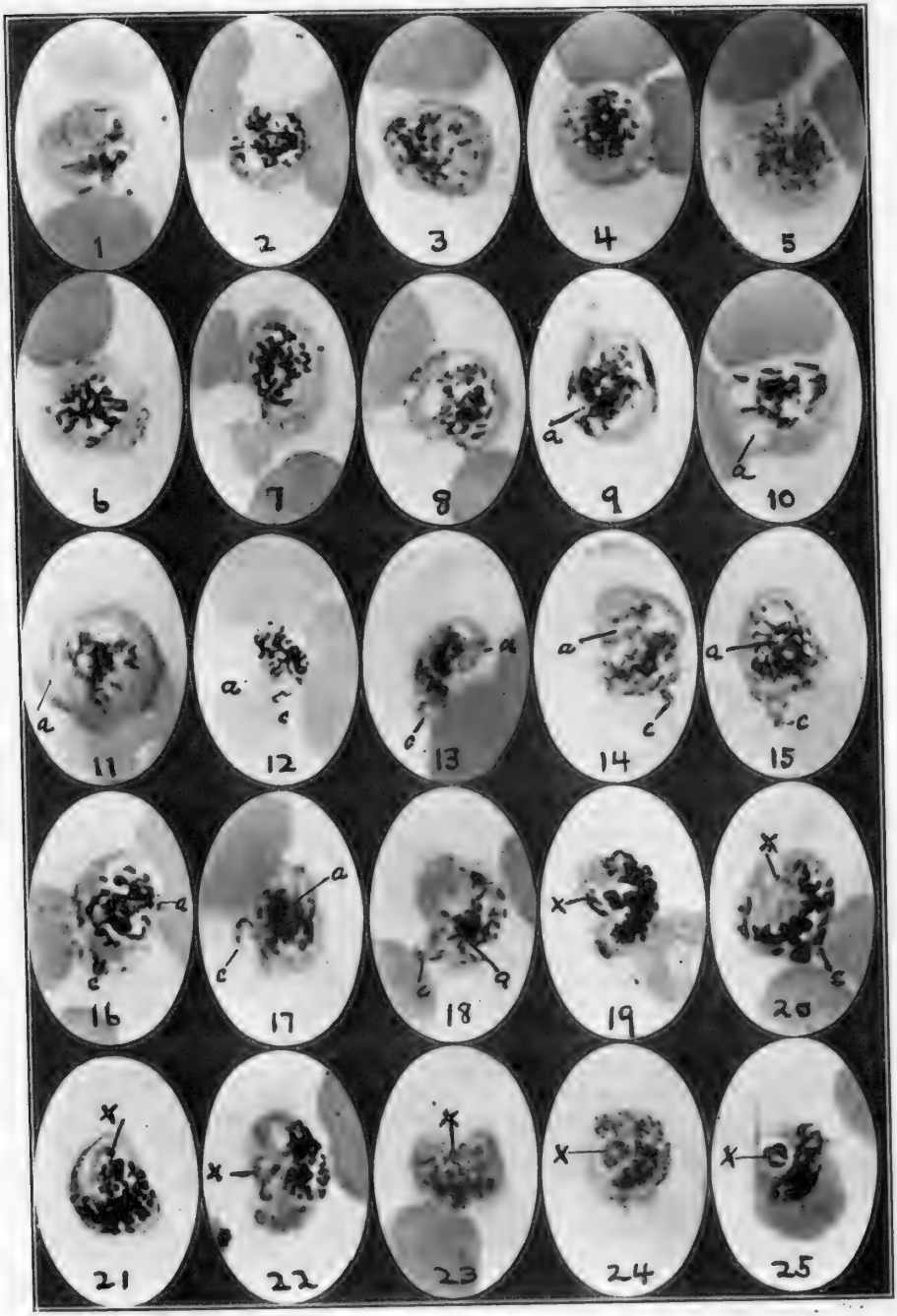
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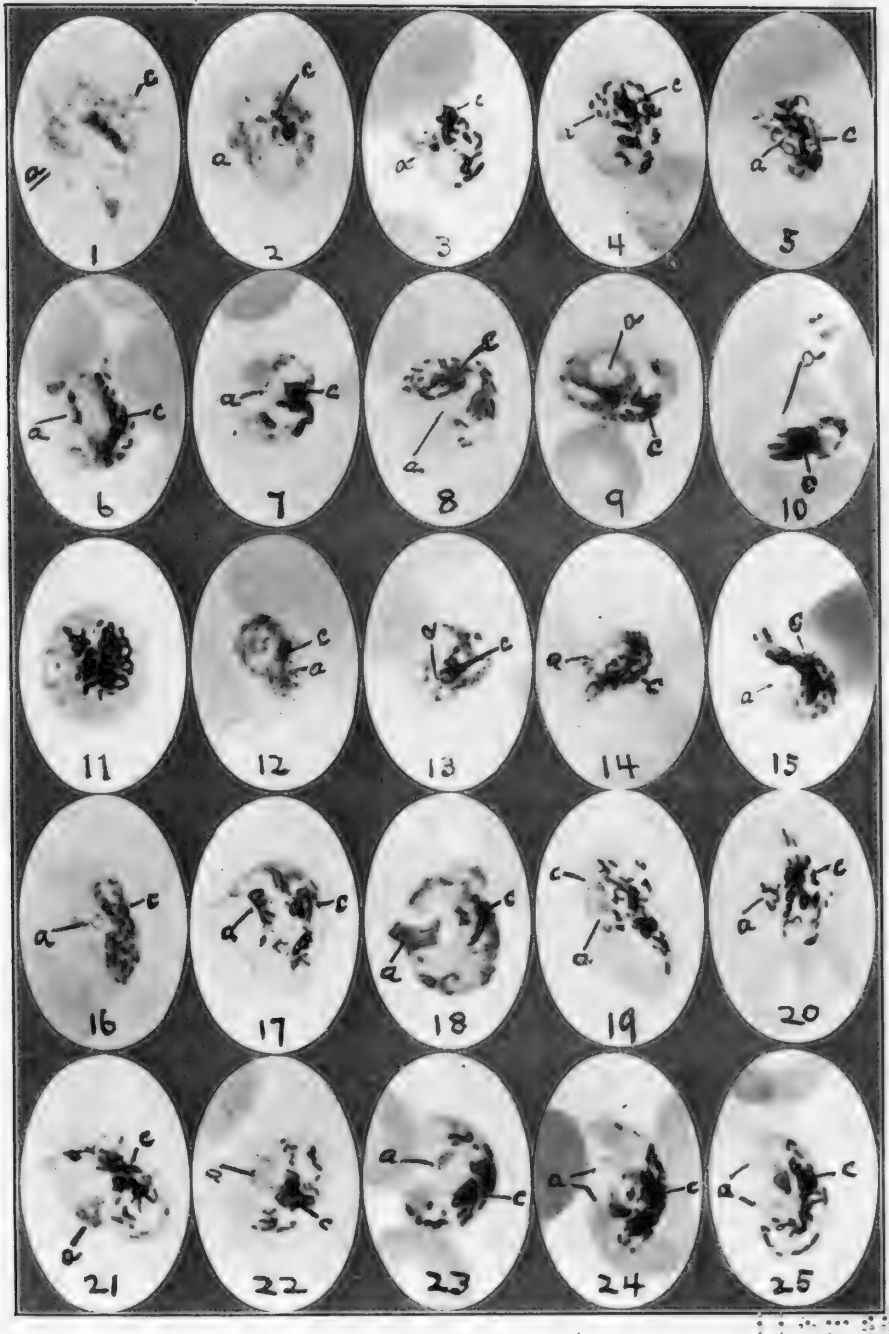
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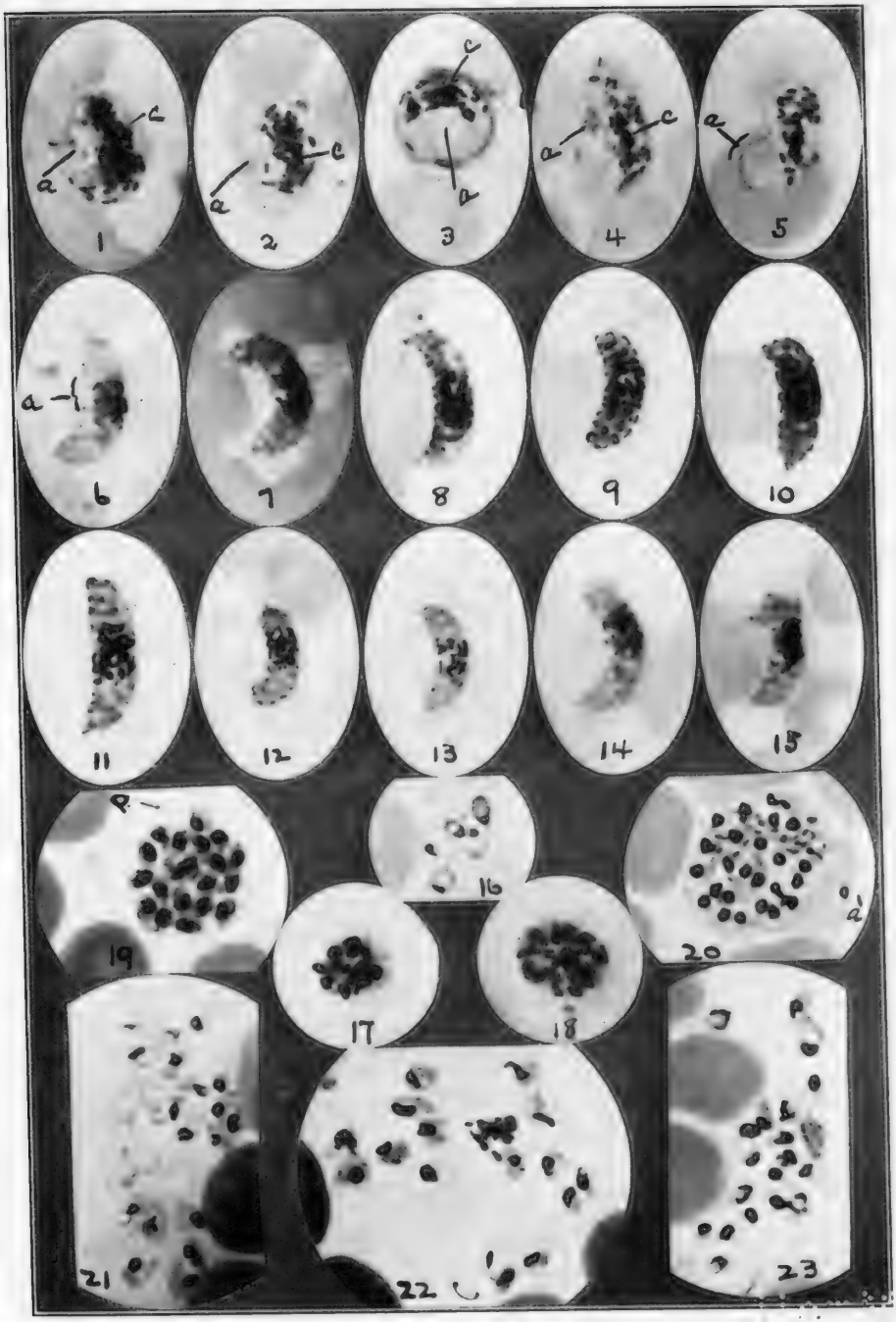
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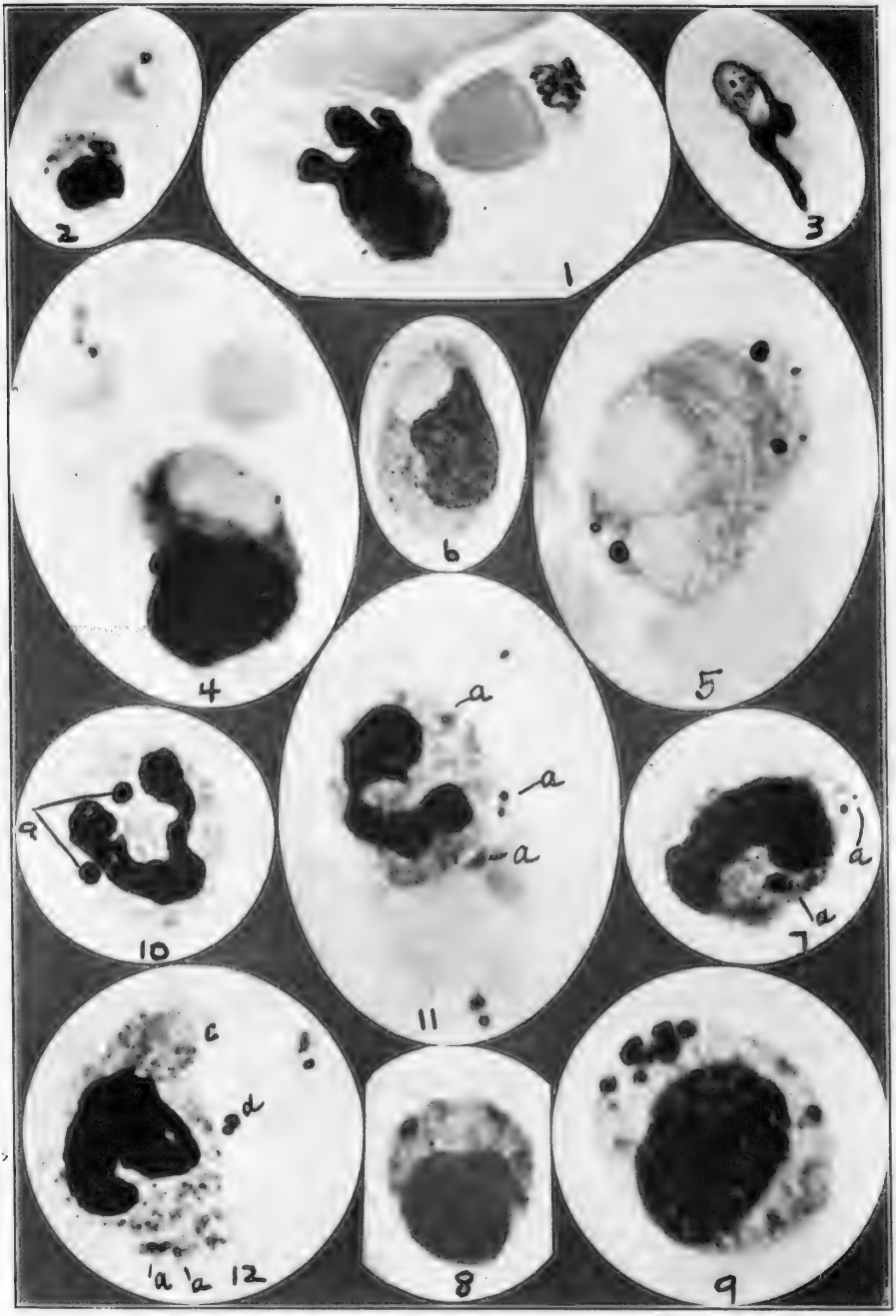

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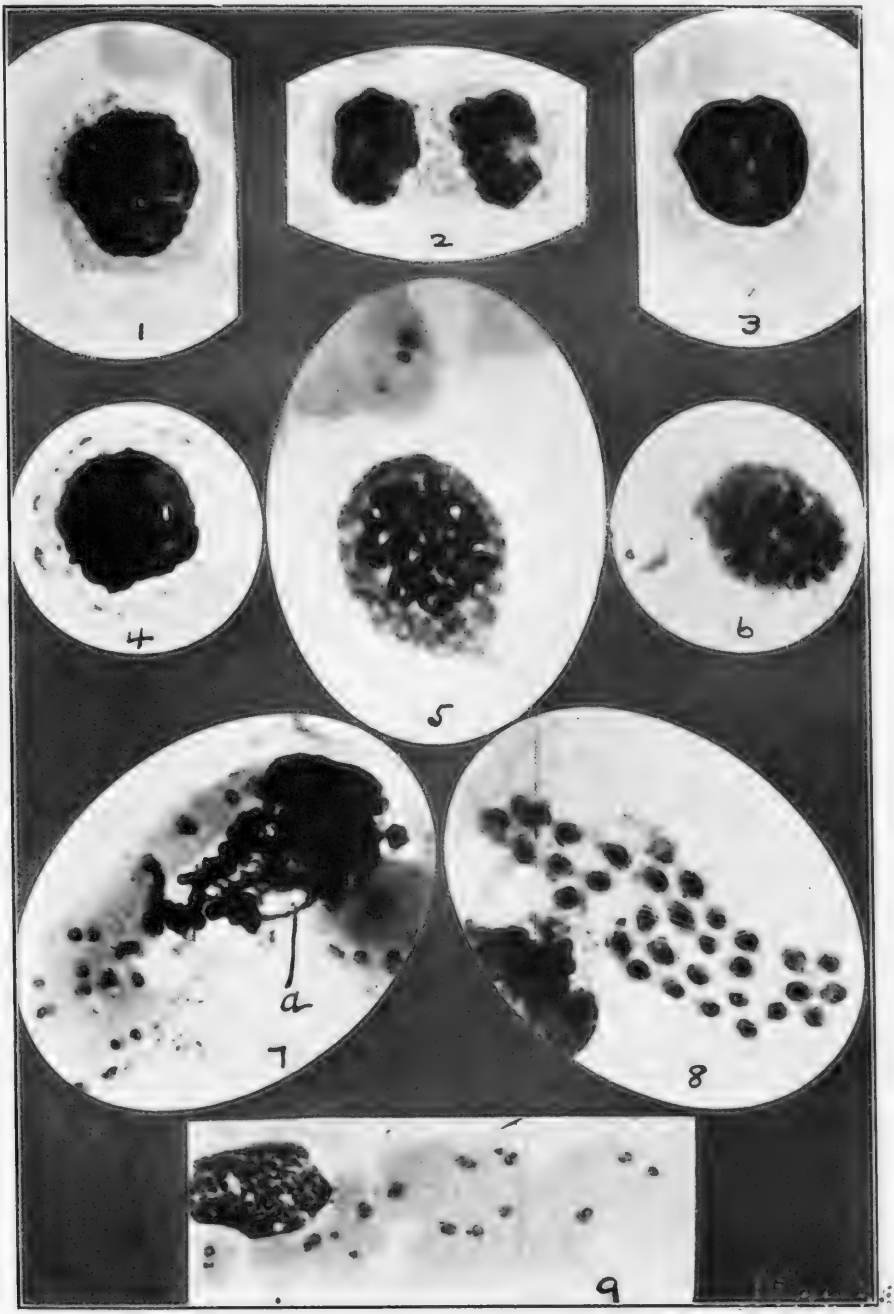
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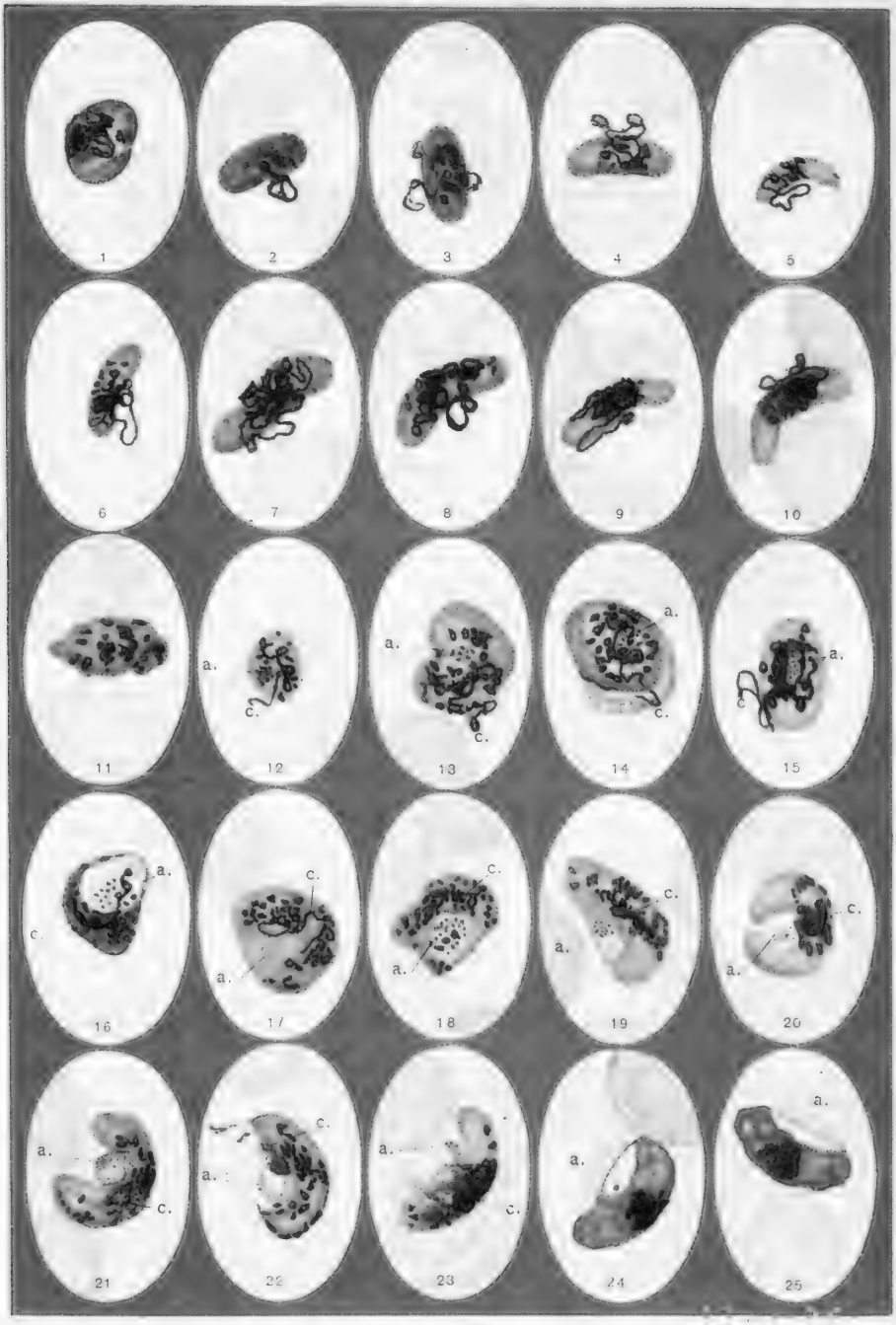
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containing at "*a*" their own nuclei in the form of granules, and flagella which have not entirely penetrated the body of the female, a small portion being seen outside at "*c*."

FIG. 16 corresponds to plate XXIV, Fig. 21. A macrogamete attached to a red corpuscle. The nucleus of the parasite can be seen at "*a*" in the form of granules stained pink in the achromatic area (here somewhat transparent so that the corpuscle upon which it lies shows through it). The flagellum can be seen as a delicate filament stained magenta, partially in the blue and partially in the achromatic portion of the parasite at "*c*."

FIG. 17 corresponds to plate XXV, Fig. 8. A macrogamete in which the flagellum "*c*" has penetrated into the blue-staining portion of the parasite. The nucleus of the macrogamete can be seen in the form of tiny granules stained pink in a small round body at "*a*."

FIG. 18 corresponds to plate XXV, Fig. 6. A macrogamete in which the flagellum has penetrated the blue portion of the parasite at "*c*." Several grains of pigment are seen overlying the nucleus of the parasite at "*a*."

FIGS. 19 and 20 correspond to plate XXVI, Fig. 4, and plate XXV, Fig. 10. Fertilized crescents showing their nuclei at "*a*," between the poles of the crescents, and their flagella at "*c*" in the body proper of the crescents.

FIG. 21 corresponds to plate XXV, Fig. 23. A fertilized crescent just opening with its nucleus in the form of tiny granules arranged in a circular mass between the poles of the crescent at "*a*." The flagellum has lost its thread-like structure and is seen at "*c*."

FIGS. 22 and 23 correspond to plate XXV, Figs. 24 and 25. Fertilized crescents showing the chromatin mass (at "*a*") described in Fig. 21; but here apparently opening out in the same manner as the crescent opens out. Note here, as in Fig. 21, the changes in the flagellum at "*c*," the thickening, loss of structure, and seemingly diminished amount as compared with the flagella seen in the other figures.

FIGS. 24 and 25 correspond to plate XXI, Figs. 10 and 13. Crescents with capsules, the red corpuscles emerging from beneath the parasites at "*a*." Fig. 24 shows a granule of pigment in the cystic portion of the parasite.

MALARIAL PIGMENT (SO-CALLED MELANIN): ITS NATURE AND MODE OF PRODUCTION.*

By W. H. BROWN, M.D.

(*From the Pathological Laboratory of the University of Wisconsin, Madison, Wisconsin.*)

The pigment elaborated by the malarial parasite during its development in the red blood corpuscle is constantly referred to in the literature as a melanotic pigment, or melanin. Yet, if one seeks authority for such an implied relation to the melanins, there seems little to warrant such an assumption. It is equally difficult to trace the origin of this conception, though it is probably referable to the work of Meckel,[1] who first described malarial pigment in the blood as a melanemia. The marked difference between this pigment and the familiar hematogenous pigments has undoubtedly aided in the perpetuation of the melanin conception of malarial pigment.

Other Pigments.—The literature on malaria contains also numerous descriptions of other types of malarial pigment, though, in reality, none of these are peculiar to malaria—occurring more frequently and even in larger amounts in conditions other than malaria. These pigments, therefore, should not be considered as malarial pigments, this term being reserved for the dark brown or black pigment which is almost, if not entirely, peculiar to malaria.

Malarial Pigment Differentiated.—The so-called malarial melanin is readily differentiated from all other pigments by those familiar with the usual descriptions of the pigment and its quite characteristic distribution.

Unfortunately, the work of Ewing[2] has introduced much confusion. Ewing describes at some length, and illustrates with most excellent cuts, granular and crystalline pigments (occurring in

* Received for publication, November 5, 1910.

[1] Meckel, *Ztschr. f. Psychiat.*, 1847, v, 198; cited by Virchow, *Virchows Arch. f. path. Anat.*, 1849, ii, 587.

[2] Ewing, *Jour. Exper. Med.*, 1905, vi, 119.

malaria as well as in other conditions with jaundice) which he considers are differentiated with "great difficulty" from the pigment elaborated by the malarial parasite. He states that in malaria one may meet with granular or crystalline pigment "not giving the Prussian blue reaction, nor dissolving in chloroform, ether, or carbon bisulphide, but dissolving in ammonium sulphide, which may have any one of the following origins:

(1) Pigment elaborated by the intracellular parasite.

(2) Hæmatoidin derived from the remnants of infected red cells.

(3) Hæmatoidin or altered hemoglobin deposited in granular or crystalline form from red cells dissolved in the plasma . . .

(4) Bilirubin or urobilin granules or crystals."

The pigments represented in groups 2, 3, and 4, he considers are rendered abnormal by fixation.

It has been impossible for me to confirm these findings. For example, in none of my preparations have I been able to detect such alterations in the bile pigments, after alcohol or formalin fixation, as to render them insoluble in the usual solvents. In groups 2, 3, and 4, I see only the precipitate, granular and crystalline, produced by the action of formalin on dissolved hemoglobin and found in greater or less amounts in nearly all tissues fixed in formalin. As Ewing states that these pigments do not give the usual reactions for bile pigments, I cannot see his reasons for considering them as such.

Although I have not investigated the nature of this formalin precipitate, Browicz[3] describes it as the conversion of dissolved hemoglobin into methemoglobin and eventually into hematin. The precipitate follows closely the solubility of the malarial pigment and for this reason, in the study of malarial pigment, tissues fixed with formalin should never be used. Nevertheless, the general features and distribution of the formalin precipitate are such as should give one no difficulty in distinguishing it from the true malarial pigment.

After what has been said, it seems hardly necessary to state that this investigation deals solely with the so-called melanotic malarial

[3] Browicz, *Virchows Arch. f. path. Anat.*, 1900, clxii, 373.

pigment and is in no wise concerned with the associated hematogenous pigments.

In the investigation of the supposed melanin nature of malarial pigment it seemed advisable to institute a comparison between this pigment and certain well known melanins. I have used several specimens of melanin from negro's skin, from the choroid, and from melano-sarcomata. The malarial tissues represent principally spleen and liver from six cases of pernicious malaria in which there were abundant deposits of pigment. The comparison made has been based largely upon bleach reactions and solubility. While different melanins vary rather decidedly in both of these respects, as is well known, they are bleached to colorless compounds with comparative ease when subjected to the action of such oxidizing agents as potassium permanganate and hydrogen peroxide. On the other hand, their resistance to solution is pronounced—even with strong acids and alkalies.

The following technique and reagents have been used in carrying out this work. Celloidin sections were used almost exclusively. A ¼ per cent. aqueous solution of potassium permanganate and a 30 per cent. hydrogen peroxide[4] were employed as bleaching reagents. More dilute solutions than these are efficacious, though these act more rapidly and damage the section but little unless used longer than twenty-four hours. Sections treated with potassium permanganate require decolorization with oxalic acid or Pal's solution. The list of solvents was not intended to be exhaustive. It included the following: saturated aqueous solution of lithium carbonate; 0.2 per cent. aqueous potassium hydroxide; alcoholic potash made by adding four parts of 1 per cent. potassium hydroxide to 100 parts 80 per cent. alcohol (0.04 per cent. alcohol potash); ammonium hydroxide, diluted one-half; ammonium sulphide; 1 per cent. hydrochloric acid in 70 per cent. alcohol (by volume); 1 per cent. hydrochloric acid ether; 5 per cent. (by volume) aqueous hydrochloric and sulphuric acids. The alcoholic potash solution is one in common use in many laboratories for removing the formalin precipitate from sections and it was accidentally noted that it was also an excellent solvent for malarial pigment. All

[4] The preparation used is the perhydrol of Merck and Company.

sections, however treated, were dehydrated, cleared, and mounted for study.

The following table illustrates the essential differences in the bleach reactions and solubility of malarial pigment and melanins.

Tissue.	Bleach reagents.		Solvents.								
	¼ per cent. $KMnO_4$.	30 per cent. H_2O_2.	Saturated Li_2CO_3.	0.2 per cent. KOH.	$(NH_4)OH$.	Alcohol KOH.	Acid alcohol.	Acid ether.	$(NH_4)_2S$.	HCl.	H_2SO_4
Negro's skin, No. 1.....	+	+	—	—	—	—	—	—	—	—	—
Negro's skin, No. 2.....	+	+	—	—	—	—	—	—	—	—	—
Negro's skin, No. 3.....	+	+	—	—	—	—	—	—	—	—	—
Choroid, No. 1.....	+	+	—	—	—	—	—	—	—	—	—
Choroid, No. 2.....	+	+	—	—	—	—	—	—	—	—	—
Melano-sarcoma, liver..	+	+	—	—	—	—	—	—	—	—	—
Melano-sarcoma, liver..	+	+	—	—	—	—	—	—	—	—	—
Melano-sarcoma, choroid.....	+	+	—	—	—	—	—	—	—	—	—
Melano-sarcoma, skin..	+	+	—	—	—	—	—	—	—	—	—
Melano-sarcoma, source unknown.....	+	+	—	—	—	—	—	—	—	—	—
Malaria, Case 1.....	—	—	+	+	+	+	+	+	+	—	—
Malaria, Case 2.....	—	—	+	+	+	+	+	+	+	—	—
Malaria, Case 3.....	—	—	+	+	+	+	+	+	+	—	—
Malaria, Case 4.....	—	—	+	+	+	+	+	+	+	—	—
Malaria, Case 5.....	—	—	+	+	+	+	+	+	+	—	—
Malaria, Case 6.....	—	—	+	+	+	+	+	+	+	—	—

Plus (+) indicates a positive change, and minus (—), no change.

So many factors influence the time required to secure complete bleaching or solution, that unqualified statements are of little value. The thickness of the section, the intensity of the color, the size of the granules, and the total amount of the pigment, all influence the time required to obtain the end reaction. Besides these factors, however, I have observed that melanins vary rather decidedly in the ease with which they bleach. In my experiments, therefore, no negative result has been recorded until the reagent has acted for forty-eight hours.

Hydrogen peroxide bleaches melanin much more slowly than potassium permanganate, and with all of my material, required four to sixteen hours to produce complete bleaching.

On malarial pigment, 30 per cent. hydrogen peroxide acts very rapidly. In some of my malarial sections there was a total disappearance of the pigment within thirty minutes, and in all cases

within two hours. The speed of reaction, in these instances, was due, not to the factors noted above, but to the nature of the pigment. On careful investigation, the change proved to be one of disintegration, no bleaching[5] being detectable.

Bleached melanin granules are readily demonstrable in stained sections, but no such granules could be detected after the action of peroxide of hydrogen on malarial pigment. Furthermore, if the sections from cases of malaria are studied from time to time during the process of dissolution, the decrease in size as well as in the intensity of the color of the pigment granules, can be observed.

Therefore, one is justified in the conclusion that, with the reagents here employed, the bleach reactions of melanin and of malarial pigment show no points in common—all melanins bleaching rapidly with potassium permanganate, while with this reagent malarial pigment manifests not the slightest sign of a true bleach reaction within forty-eight hours.

If we consider the question of solubility of malarial pigment and of melanin, the difference is equally pronounced. Neither substance is soluble in alcohol, ether, or chloroform. The resistance of melanins to solution by such reagents as were employed in these tests is not at all surprising. On the other hand, in view of the fact that Ewing and some others record only one solvent for malarial pigment, namely, ammonium sulphide, it is quite surprising to find such a list of substances that will dissolve this pigment with comparative ease, even in extreme dilution.

My own results, in this respect, are closely in accord with those reported by Carbone,[6] who pointed out the parallelism in the solubility of malarial pigment and of hematin. This parallelism of solubilities led me to make a spectroscopic examination of the malarial pigment.

Solution of Malarial Pigment.—The difficulty and possible source of error attendant upon such an examination was to obtain a solvent which, while dissolving the malarial pigment from fixed

[5] A distinct bleaching of the malarial pigment may, however, be seen to precede the disintegration provided a 3 per cent. solution of the hydrogen peroxide is used instead of the 30 per cent. solution employed in these experiments.

[6] Carbone, *Gior. d. r. Accad. di med. di Torino,* 1891, series iii, xxxix, 901.

tissues, would not affect the hemoglobin of the blood corpuscles. For this purpose, I employed the solution of alcoholic potash described above. In preparing the solution of malarial pigment, a piece of spleen (one cubic centimeter) fixed in Zenker's fluid was dried and ground in a mortar. Ten cubic centimeters of the alcohol potash, previously described, was poured over the powdered spleen and allowed to stand for twelve hours in a tightly corked flask and then filtered. Bile pigments, when present, should be extracted before dissolving the malarial pigment, though they may be separated subsequently. The solution thus obtained was of a brown color. As a control, a piece of spleen of the same size from an acute splenic tumor and a blood clot of the same size, both fixed in the same way as the malarial tissue, were treated in identically the same manner. As the filtrate from these was perfectly clear, the strength of the alcoholic potash was increased by the addition of two drops of 10 per cent. potassium hydroxide and this was allowed to act for twenty-four hours, after which the filtrate still showed no trace of color. From this, it was absolutely certain that the color in the filtrate from the malarial tissue was not derived from the red blood corpuscles or dissolved hemoglobin, but was referable to the malarial pigment in solution.

Spectroscopic Examination.—The solution of malarial pigment, when subjected to spectroscopic examination, showed a single band starting sharply at the D line and extending to the left. The left border was very indistinct, gradually shading into the red between C and D. On acidifying the solution with a drop of hydrochloric acid, the color became redder. This solution also presented a single banded spectrum. In this case, however, the band was moved markedly towards the left, the spectrum undoubtedly being that of acid hematin. On further treating this solution with Stoke's reagent, it assumed the characteristic color of a hemochromogen solution. Spectroscopically, it showed the two perfectly characteristic bands of hemochromogen in the green, the left band being much the darker and more distinct. On shaking with air, the color changed and no absorption bands could be detected. On again adding Stokes' solution, the two bands reappeared. A portion of the original solution added to concentrated sulphuric acid failed to

show any absorption bands, but when the solution was evaporated to dryness and the greenish black residue added to the concentrated acid, a spectrum with two well defined bands was obtained. The fainter and narrower band was situated just to the left of D, while the darker and broader band lay between D and E—the characteristic spectrum of hematoporphyrin.

The spectroscopic examination of malarial pigment confirms the results obtained by Carbone in 1891 and leaves no question as to the hematin nature of this pigment. It is rather remarkable that this conception of malarial pigment seems to have gained no confirmation or acceptance. In fact, the author finds this most excellent work of Carbone almost entirely overlooked or misquoted.[7]

When we consider the marked difference in the bleach reactions and solubility of melanins and malarial pigment, it seems almost impossible that the two substances could be placed in the same class. The statements found in the literature are not entirely in accord with my own findings in these particulars. For example, Thayer[8] speaks of the decolorizing of malarial pigment by alkalies. This is not a true bleach reaction but a solution. Again, Ewing states that the pigment "long resists the action of strong acids and alkalies." I cannot agree with this statement, for, while relatively strong aqueous solutions of sulphuric and hydrochloric acids do not dissolve malarial pigment, very dilute alcoholic and ether solutions of these acids are good solvents and alkalies in either aqueous or alcoholic solution dissolve malarial pigment even in extreme dilution.

So much emphasis has been placed upon the negative iron reaction of malarial pigment that I feel I cannot leave the question of the nature of the pigment without mentioning this reaction. It must be remembered that the iron reaction with potassium ferrocyanide and hydrochloric acid, as shown by hemosiderin, is the reaction of a proteid-iron compound in which the Prussian blue is largely deposited in the proteid matrix. Hematin is not such a

[7] During the progress of this work, the author relied upon reviews of Carbone's paper for his information of that investigator's work. The original work of Carbone was not obtained until this paper was in manuscript, and several changes have been necessary to give him full credit for his results.

[8] Thayer, Lectures on the Malarial Fevers, New York, 1897, p. 240.

compound, and on *a priori* grounds we should not expect the same type of reaction but rather the reaction of a soluble iron salt in which the Prussian blue is not fixed *in situ*. Milner[9] goes as far as to state that hematin does not give a microchemical reaction for iron. It is certainly not demonstrable by the usual technique.

The Iron Reaction Obtained.—If the test for iron in a celloidin section containing malarial pigment is made on a slide with a mixture of ferrocyanide and acid alcohol (2 per cent. or stronger), and if the cover is sealed with vaseline or paraffin to prevent evaporation, a spreading halo of Prussian blue can be demonstrated about many of the pigment granules. This is more pronounced after the preparation has been gently warmed or allowed to stand for some time. If there is any suspicion that this reaction is due to a collar of hemosiderin, such a possibility can be eliminated by the removal of all iron from the hemosiderin with Pal's solution or a 1 to 2 per cent. solution of oxalic acid allowed to act for twelve hours; usually a shorter time is sufficient and this treatment does not affect the malarial pigment. A preliminary oxidation, preferably with hydrogen peroxide, will facilitate the iron reaction. At all events, the residue from a solution of malarial pigment, neutralized with hydrochloric acid and evaporated to dryness yields when incinerated, an iron-containing ash.

Turning now to the mode of formation of malarial pigment and the possible biochemical factors concerned in its elaboration, the problem seems rather easy of solution. It is well known that many protozoa possess proteolytic enzymes analogous in their action to pepsin or trypsin. Pepsin and trypsin, acting upon hemoglobin, split it into a proteid (globin) and hematin. At present, no one doubts that malarial pigment is produced from hemoglobin by the malarial parasite. From these facts and from what we know of the nature of the pigment in question, it is only a logical assumption that the malarial parasite contains some proteolytic enzyme, analogous to pepsin or trypsin, capable of splitting hemoglobin into proteid and hematin, the proteid being further metabolized in the economy of the parasite, and the hematin being merely a waste product, accumulating as the parasite develops, to be finally extruded on the segmentation or disintegration of the parasite.

[9] Milner, *Virchows Arch. f. path. Anat.*, 1903, clxxiv, 475.

The ultimate fate of the malarial pigment is uncertain. That it remains for years in the spleen and other organs in which it is deposited indicates strongly that the human organism possesses no adequate means for its disposal. This fact is of much importance in its bearing on hemoglobin metabolism, indicating that hematin is not formed in any considerable amount as an intermediate product in the production of bile pigments, as is at present taught.

For years, the question of the extra-parasitic production of malarial pigment has been under discussion. Hemosiderin, present in the tissues, has been suggested as a possible source for such pigment. While this question has never been absolutely settled, it seems to the author quite certain that such a mode of formation plays no important rôle in the pigment production. The occurrence of black pigment in the centre of hemosiderin masses in malaria has added strength to the extra-parasitic hypothesis. Such pictures have been observed in a few instances in hemosiderin deposits in other conditions and the significance has been indicated by the author[10] in a previous publication. It is in reality a conversion of hemosiderin into hematin. As this rare change occurs in conditions other than malaria, I consider it of no particular importance, but only a concomitant process which probably has nothing to do with the production of malarial pigment. More frequently the black pigment is merely a nucleus about which the hemosiderin is deposited.[11]

In conclusion, it seems hardly necessary to state that I do not assume that the black malarial pigment is pure hematin. There are doubtless impurities, although I believe nothing of sufficient consequence can be demonstrated to warrant the perpetuation of the misnomer of melanin for a pigment that is in reality hematin.

SUMMARY.

1. Two important methods for the study of malarial pigment are described.

(*a*) A method for obtaining a solution of malarial pigment from fixed tissues without the removal of a trace of hemoglobin from the red blood corpuscles.

[10] Brown, *Jour. Exper. Med.*, 1910, xii, 623.

[11] Neumann, *Virchows Arch. f. path. Anat.*, 1900, clxi, 422.

(*b*) A method for obtaining an iron reaction in malarial pigment.

2. By comparing the bleach reactions and solubility of melanins and malarial pigment, the dissimilarity of the two classes of pigments has been demonstrated.

3. The spectroscopic examination of a solution of malarial pigment proves conclusively that the pigment is hematin.

4. It is suggested that the action of a proteolytic enzyme of the malarial parasite upon the hemoglobin of the red blood corpuscle is the most probable mode of elaboration of malarial pigment.

5. The difficulty with which the human organism disposes of malarial pigment indicates that the production of hematin cannot be considered as a normal intermediate process in the formation of bile pigments from hemoglobin.

My thanks are due to Dr. G. H. Whipple, of the Johns Hopkins Hospital, for supplying me with a part of the tissue used in this investigation, and to Dr. A. S. Loevenhart, of the University of Wisconsin, who has kindly aided me with the spectroscopic work and confirmed that part of the findings here reported.

Christian Archibald Herter

b. 1865 d. 1910

At a meeting of the Association of the Staff of the Rockefeller Institute held February 10, 1911, the following Minute was adopted:

The members of this association of scientific workers in the Laboratories and the Hospital of the Rockefeller Institute for Medical Research desire to record their sorrow on the loss of their honored associate, CHRISTIAN ARCHIBALD HERTER, *M.D., Member of the Institute, Member of the Board of Scientific Directors, and Physician to the Hospital. Dr. Herter's death is the first to break the ranks of this community of men joined closely together by their common devotion to medical and biological research. As a member of the original Board of Directors, Dr. Herter was intimately concerned with the careful and far-sighted planning which has resulted in the present organization of this institution. His part in the direction of its activities was enlightened by his own versatile and productive work in the fields of physiological, pharmacological, and chemical research. Sharing the belief of his colleagues that a research hospital was essential to any well-rounded plan for investigating the diseases of human beings, his vision of what constituted an adequate building, equipment, and personnel was largely responsible for the liberal scale on which the work of the Hospital has now been organized. Upon the opening of the Hospital he threw himself with characteristic ardor and self-sacrifice into his chosen lines of clinical investigation. Utterly refusing to surrender to an infirmity which would have blighted both the moral and physical energy of an ordinary man, he held to his work until the very week of his death, leaving an example of heroic self-forgetfulness and devotion to science that will be long remembered in the annals of the Rockefeller Institute.*